HUMAN INFANT OLFACTION:- RESPONSES TO FOOD ODOURS MEASURED BY BRAIN ELECTRICAL ACTIVITY MAPPING (B.E.A.M)

IN TWO VOLUMES.

VOLUME ONE

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SUBMITTED FOR THE DEGREE OF PhD.

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September 1990

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ACKNOWLEDGEMENTS

The research described in this thesis was generously funded by the **Wellcome Trust**, who I wish to thank for their financial support in the form of the Wellcome Trust Prize Studentship. I would like to gratefully acknowledge the constant support, encouragement and unstinting material assistance provided by my supervisor, Dr. Steve Van Toller. His vast knowledge and expertise in the field of olfaction was always available to me and willingly provided. The assistance and cooperation of the following persons and institutions is also gratefully acknowledged (listed in alphabetical order):

Dr. G. Collis.
Mr. K. Halstead.
Dr. P. Kaplan.
Prof. J. Steiner.
Dr. J. Werner.

Coventry Health Authority.
Coventry Maternity Hospital (Ante-Natal Clinic Staff).
Cow & Gate Limited.
Densa Limited.
Henley's Medical Supplies Limited.
Neuroscience (UK) Limited.
Psychology Departments, University of Warwick, U.K., and University of Colorado at Boulder, USA., and their technical staff.

Last, but not least, I wish to acknowledge the constant support and encouragement of Kathryn Ingham; partner in Life, the Universe and Everything....

DECLARATION

The material contained in this thesis has never been previously submitted for any higher degree and represents the author's own work. Some pilot experiments summarised at the beginning of Chapter 4 were carried out at the University of Colorado. This work was preliminary and formed no part of studies submitted for the degree of PhD. All remaining experimental work described in this thesis was carried out in the Department of Psychology, University of Warwick.

SUMMARY

This thesis addresses the area of human infant olfaction, which has hitherto been a somewhat neglected area in psychology. A review of the available literature showed that a number of different experimental approaches to infant olfaction could be identified. It was concluded from this literature review that infants display a degree of olfactory competence in the weeks after birth. These previous findings are discussed in the light of a model concerned with odour significance. This model is systems-based and suggests an explanation for the apparent olfactory competence of human infants in the first weeks of life. It is argued that this may derive from pre-natal exposure to odorants and consequent acceleration of maturation in the foetal olfactory system.

The experiments reported in this thesis concentrated on the cortical reactions of infants to a small range food odours. These reactions were plotted by means of a technique involving electro-encephalography. This technique involves a computerised imaging system which summarises cortical potentials from twenty-eight locations on the scalp and is known as Brain Electrical Activity Mapping (BEAM). It is believed that this is the first time that this method has been used to examine responses to odour in human infants. This study also involved the use of a special low ambient odour testing environment. A parallel study used respiratory plethysmography to test odour response. The major findings of the BEAM research are as follows:

1) The BEAM technique has been shown to be a practical method in the psychophysical measurement of cortical responses to odour in the human infant.

2) Human infants at the age of three months show a pattern of cortical activity in response to a small range of food odours.

3) There is evidence that a limited area of the infant brain is responding to these odours.

It was argued that these findings lent some support to the model described above. However, similar findings to that of the BEAM work were not shown by the respiratory plethysmography study. This was explained by problems in data handling techniques. It was concluded that the BEAM method could be used to further knowledge in the area of infant olfactory response. Possible improvements to the experimental technique were discussed which would allow testing of infant response to maternal odour. Such future work could shed considerable light on the role of olfaction in the early infant-mother relationship.

PREFACE

It should be noted that throughout this thesis, the terms 'infant' and 'baby' have been operationalised. 'Infant' refers to children up to and including the first three months, and 'baby' is taken to refer to the first year of life. In line with current publishing practices to avoid sexist language, all references to one gender may be taken to include both. All scientific units of measurement are given in SI units. This thesis follows the presentation conventions of the British Psychological Society (BPS) cited by: Sternberg, R.J. (1988) **The Psychologist's Companion (2nd. edition)**. England: Cambridge University Press/BPS.

Each chapter is divided into sections and paragraphs. Sections are generally identified by bold-faced, underlined headings (e.g. **Method**). Paragraphs are numbered according to the following convention. The first number refers to the chapter. The second number refers to a concept, area of discussion or topic. A new number is generally given whenever one of these is introduced. The final number refers to paragraphs linked with the second number. Hence, paragraph 4.2.4. defines Chapter 4, topic 2, paragraph 4.

Introduction to Thesis

"If I were a young researcher interested in making a career in science, especially in the area of neurobiology and the mechanisms of the human brain: and if I was looking around for a field about which nobody knows much of anything and if I was ready, as all good researchers must be ready, to gamble on a career in science, I would pick the problem of olfaction - and I would count on a professional lifetime of one surprise after another." Lewis Thomas, cited in Green (1988).

"In the domain of human perception, the "chemical senses" (taste and smell) are clearly the poor relations of research in neuroscience." Bruyer (1988).

"The omission of taste, touch and olfaction... is.... an accurate reflection of the disproportionate concern which has been afforded to vision and audition in studies of infancy... Such an imbalance would detract from a comprehension of functioning at any stages of development, but may be particularly distorting with regard to our understanding of the world of the infant." Türkewitz (1979, cited in Schaal, 1988a)

"The status of the chemical senses at birth has become better understood; perhaps the real challenge now is to understand events at weaning, when the child encounters a wealth of new experience from these senses." Crook (1981).

Preamble

Much of the human infant sensorium has been investigated by psychologists in the last hundred years. A considerable amount is known about infant visual, auditory and somatosensory perception although, of course, much remains to be learned (Werner & Lipsitt, 1981). Despite these advances, a whole area of infant sensory experience has been relatively neglected. Human infant olfaction remains largely a *terra incognita*. Indeed, the study of human

olfaction in general has been under-emphasised by science. As Lewis Thomas described it: *"a field about which nobody knows much of anything"*. A good test of this contention is to consult most general textbooks on sensory psychology published in the last 40 years. The chances are that human olfactory perception will merit a sub-section of a chapter, or even as little as a paragraph. The empirical evidence is likely to be sparse, even inaccurate (though there are exceptions). The section usually closes with a dismissive statement that nothing much is known about the area, with the clear implication that it is not important anyway.

However, interest in the 'chemical senses' has started to grow in the last few years. The formation of research organisations like the European Chemoreception Research Organisation (ECRO), Association for Chemoreception Sciences (AChemS) and Japanese Association of Smell & Taste Scientists (JASTS) has helped to promote this growth of interest in a hitherto neglected field of human experience. However, whilst the field of adult olfaction has seen a growth of interest, the area of human infant olfaction has lagged behind. The principal aim of this thesis has been to play a small part in improving knowledge about this area.

Aims of the thesis

The goals of this thesis were essentially four-fold and are described below in the order in which the chapters appear. The first aim was to try and describe and integrate what little is known about the biological development of human infant olfaction. This is to be found in Chapter 1. The purpose of this chapter was to provide a context for the research programme by giving an understanding of some of the biological mechanisms involved in infant olfaction. Since the research involved addressing broadly 'psychobiological' events , description of the biological substrates provided a background for the understanding of these events.

The second aim was to examine the literature in the field of infant olfaction, delineate trends and schools of thought and trace the development of thinking in the area. This material comprises Chapter 2. The point of this chapter was to further enhance the

context of the research programme. By tracing the evolution of the various research trends, mainly in this present century, the rationale for the experimental work described in this thesis could be better established.

The third aim of this thesis was to describe a model which might account for the relative olfactory competence displayed by infants. This competence has been demonstrated in many of the studies described in Chapter 2. The model is based on 'systems thinking' and proposes that pre-natal olfactory influence may occur in the human infant. The model is biologically based and relates to the substrates described in Chapter 1.

The fourth and ultimate aim was to describe a number of experiments involving objective measurement of cortical and other psychophysical activity in young human infants presented with food odours. This mainly methodological aim is encompassed in Chapter 4, which comprises the bulk of the thesis. The use of a relatively new technique, which became available only in the 1980's (Brain Electrical Activity Mapping, or BEAM), is evaluated in the testing of infants. The main aim was to examine whether the BEAM technique represented a usable tool for investigating infant olfactory response. This was confirmed, despite considerable technical problems.

The final chapter draws together the various strands described above. The main conclusions of the experimental work are discussed in the light of the original hypotheses discussed at the beginning of Chapter 4. Future directions are examined and some likely courses for this kind of research are suggested.

CHAPTER 1
ASPECTS OF ANATOMY, PHYSIOLOGY AND ONTOGENY OF OLFACTION IN *HOMO SAPIENS*

Introduction

1.1.1. This chapter aims to summarise some of the knowledge about the anatomy, physiology and neurological development of the human olfactory system. The reason for doing this is to set a context for the empirical research described elsewhere in this thesis. By attempting a synthesis of what is known, it is hoped that a clearer picture of the biological substrate to infant olfaction will emerge.

1.1.2. Human olfaction is an immense area, which is very diverse in nature. A full understanding of the olfactory system in *Homo sapiens* is likely to derive only from a multi-disciplinary, eclectic approach. This is because each level of the system contains elements relevant to several disciplines. It is not at all clear what the boundaries and degree of overlap of each speciality are. For example, the physical nature of the odorants is partly the domain of organic chemistry, whilst their olfactory transduction falls within the expertise of biochemists and molecular biologists. At a 'higher', cortical level, odour perception may one day be explained by electrophysiologists, neuroanatomists and psychologists. This chapter attempts to combine a little of the knowledge of each discipline, taking the view that any detailed approach to olfaction requires evidence from living systems wherever possible.

1.1.3. This raises problems of obtaining evidence, when one is interested in human olfaction. As described below, one of the major problems in studying this area is its inaccessibility. This is due firstly to physical reasons and secondly to ethical constraints. Until recently, the surgical techniques did not exist to perform *in vivo* biopsy of olfactory tissue in human beings (Lovell *et al*, 1982; cited in Moran, Rowley, Jafek & Lovell, 1982). Now that they do, it is hoped that more information about the human olfactory system will become available. However, this may await assays that can deal with the minute amount of living tissue that can currently be obtained by biopsy. The techniques involving odour deprivation, cortical ablation

and autoradiography that have yielded so much information from animals are never likely to be replicated in humans, not merely because of the obvious ethical problems. Because of this, psychology has a major part to play in understanding Structure-Activity relationships.

1.1.4. Biochemists have long used animal models to try and comprehend how structure in the olfactory system is related to function. This can be done by manipulating the animal's ambient odour environment, then sacrificing the animal to examine the often subtle changes in the olfactory biochemistry. Clearly, such experiments are not possible in humans. Hence, scientists have to rely upon behavioural changes to understand the effects of odour. Psychology clearly has a major part to play here. With the use of psychophysical and psychometric techniques, insight into structure-activity relationships can be inferred.

1.1.5. Because much of the work in the area of olfactory anatomy and physiology has been done with rodents and other small mammals it is always difficult to know to what degree cross-species generalisations are valid. The position taken in this thesis is that, although animal models provide a framework for understanding human olfaction, cross-species generalisations to humans need to be cautious. Because of this, animal work will only be referred to when there is an absolute lack of human evidence.

1.2.1. This chapter is subdivided into the following sections:

Section 1: gross anatomy, micro-anatomy and ultrastructure of the human olfactory system.

Section 2: physiology of the human olfactory system.

Section 3: development of the human olfactory system.

Each of these sections will concentrate on those areas which are relevant to the topic of this thesis. The information in the above sections is largely taken from the excellent descriptions of anatomy

and physiology that can be found in some recent works; notably Barr & Kiernan (1983), Castellucci (1985), Macrides & Davis (1983), Moulton (1978), and Van Toller, Dodd & Billing (1985). Specific discussion of comparative micro- and ultrastructure is gleaned, unless otherwise identified, from the article by Moran *et al* (1982). Histological data from infants comes mainly from the 1984 study by Nakashima, Kimmelman & Snow.

Section 1
Gross anatomy and micro-anatomy of the human olfactory system.

1.2.2. It should be made clear that relatively little anatomical work has been done on the human infant olfactory system. This is probably due, in part, to the ethical problems of obtaining the necessary autopsy material. These prefatory remarks should serve as a *caveat* over the problem of comparing the adult system with that of the infant. The major differences are probably those of scale and size. A paper by Laitman & Crelin (1976) contains photographs of autopsy material as well as airway casts of infants, children and adults. The major differences in size and shape can be clearly seen. The anatomical relationships of the structures are therefore different. This has been noted by Cole (1982), amongst others. Differences in shape and size mean that the flow patterns of air, and hence odorants, in the nasal cavity are probably quite different in the human infant from those found in adults. Very recent evidence suggests such differences between children and adults (Mennella & Beauchamp, 1990a). These may have implications for chemoreception in infants. Nonetheless, the scarcity of cadaver material and consequent lack of published work on the normal anatomy seriously hinders investigation of the human infant olfactory system. For this reason, this section will concentrate on evidence gleaned mainly from adult subjects, with comparative anatomical input from infant studies where possible.

Gross Anatomy.

1.2.3. As with all sensory systems, structure subserves function and one of the nasal cavity's functions is to bring inspired air and odorants into contact with olfactory receptor cells. The

airflow patterns in the human nasal cavity have been mapped, and it has been found that the anatomy of the nasal cavity serves to perturb the mainly laminar flow of inspired air in the anterior portion (Proctor & Swift, 1977; cited in Cole, 1982). Because of this, turbulence is created, which is necessary to physiological functioning. This is stated by Cole (1982, page 173):

> *"The energy required to promote and maintain the disturbed state of respiratory air is not a waste of energy, these characteristics of airflow are of physiological importance. If they did not occur, a boundary layer of air would insulate the main airstream from the mucosa".*

1.2.4. Clearly, this would have implications for the diffusion of odorous molecules into the mucosa of the olfactory neuroepithelium, which is the necessary precondition for transduction and chemoreception (Berglund & Lindvall, 1982, page 281). Stuiver calculated that, in any case, only 2% of the molecules entering the nasal cavity actually make contact with the olfactory receptors (Stuiver, 1958; cited in Gescheider, 1985). However, this investigator may not have taken into account the turbulence of the airflow which would predispose to a greater number of receptors being stimulated (Saito & Nashihata, 1981, mentioned below).

1.2.5. The human nasal cavity subserves several functions, only one of which is concerned with olfaction. For example, according to Eccles (1982):

> *"The nose therefore acts as a filter, a humidifier and a heat exchanger... the nose functions as an air conditioning system"* (page 192).

Hence the anatomy has evolved to cope with these functions, as well as olfaction. The heavy vascularisation of the nasal cavity and the presence of mucous-producing goblet cells are an example of structure subserving function. According to Tos (1982), the distribution of these glands is variable throughout the nasal cavity. He suggests that one of the factors influencing this may be the

variations of airflow within the cavity. Tos further states that the density of goblet cells is lower in infant and children than in adults (Tos, *op.cit.*). The reasons for this may be connected with the differences in airflow patterns caused by the differences in anatomy, mentioned above, in younger subjects.

1.2.6. In the human infant, the same 'air conditioning' functions are necessary; probably to a greater degree than in the adult. The reason for this is the much smaller lumen of the nasal and bronchial passages which need to be kept patent at all costs to allow unimpeded respiration. This is why upper respiratory tract infections in babies, especially those which cause oedema of the mucosa (croup, for example), may be more serious than in adults. Indeed, as stated by Cole (1982):

> *"Partial nasal obstruction has been suggested as a cause of infant cot deaths"* (page 165).

This is probably because, as noted by Cole: *"Infants are obligatory nose breathers"* (*op. cit.*, page 165). This author contends that it is not possible for infants to use the mouth for breathing until the age of 5 to 6 months (*op. cit.*, page 164). If this is so, then the infant olfactory system will, *ipso facto*, receive continual stimulation during the first months of life. However, work on models of the adult nasal cavity suggests that, unless actual 'sniffing' is employed, very little odorant reaches the receptors (Stuiver, 1958; cited in Gescheider, 1985). Infants have clearly not yet learned this ability in their first few months. Nonetheless, as no empirical work appears to have been done on the airflow patterns in the infant nasal cavity, it is unknown approximately how much of any odorant is likely to reach the receptors. Hence, it is not yet possible to either confirm or refute Stuiver's work. However, a contrary view is taken by Saito & Nashihata, (1981; cited in Schaal, 1988a). These workers suggest that the higher airway resistance induced by the narrower airway passages in the infant nasal cavity may actually improve aeration of the olfactory neuroepithelium. This was suggested as early as 1932, by Livingstone (Livingstone, 1932, page 64). This may explain the

generally low detection thresholds demonstrated by infants (Rovee, 1969; Balogh & Porter, 1986)

1.2.7. Turning to the olfactory region, the structure of this area is arranged so as to receive inspired odorants on the incoming airflow, via the olfactory cleft. Hence it is necessary to know something of the physical properties of the nasal cavity, in order to understand how odorants arrive at the olfactory mucosa. The 'aerodynamic' properties of the nasal cavity have been investigated, as mentioned above. The airflow is a function of size, shape and mucosal conditions as well as inspiratory velocity. In the adult, the nasal mucosa consists of about 160 cm^2, with a nasal cavity volume of approximately 20 ml. The cross-sectional area is about 130 mm^2. This results in a transit time of any given portion of inspired air of about $^1/_{20}$ of a second, during quiet breathing. This is partly due to the shape, and hence resistance of the anterior nasal cavity, permitting an airflow velocity of up to 18 metres/second in the adult. All these figures (Cole, 1982) are for adults, as no information is readily available for infants. This is despite a study by Nakashima *et al* (1984) which studied late foetal material. However, the main point is that the inspired airflow, laminar at the external nares, becomes turbulent within the nasal cavity. This ensures dispersion and mixing of any odorants which reach the nasal cleft. In the adult human, this is located at the most superior portion of the nasal cavity and is highly inaccessible to *in vivo* observation or biopsy. As stated by Moran *et al*, (1982) this is:

> *"A tiny 1.5 mm crevasse between closely apposed nasal bones (turbinates and septum)... that lies within the recesses of the skull some 7 cm deep to the skull"* (page 722).

In human infants, no figures are available. However, it is reasonable to assume that the olfactory cleft is considerably smaller because of the overall smaller skull size, even though the infant skull is proportionately larger than the adult. This may well have implications for the amount of odorant that actually stimulates the receptor cells.

1.2.8. A further difference between the adult and foetal/infant nasal cavity is the vomeronasal (Jacobson's) organ. This can be seen in the foetal and infant nasal cavity, but rarely in the adult according to at least one worker (Humphrey, 1940). However, Johnson *et al* claim that it can be found in a large proportion of adults (Johnson, Josephson & Hawke, 1985; cited in Schaal, 1988a). The vomeronasal organ has excited anatomists for a long time, because of its apparent resemblance to a similar organ found in other species, such as marsupials, ungulates, reptiles, rodents and carnivores (Moulton, 1978). For example, it is believed that the *nervi terminales* serves to innervate and perhaps subserve an olfactory function in the vomeronasal organs in these species (Eccles, 1982). Certainly, these nerves exist in *Homo sapiens* and run alongside the olfactory tracts. However, since the vomeronasal organ is considered vestigial, or even absent in adults, the function of the *nervi terminales* is unclear (Moulton, 1978; Widdicombe & Wells, 1982).

1.2.9. The vomeronasal organ has been described in the human foetus, as late as the seventh gestational month (Nakashima *et al*, 1984) and even in neonates (Read, 1908; Peter, 1925; cited in Schaal, 1988a). These structures are found bilaterally on the anterior part of the nasal septum. They are described as:

> *"tubular...* [and] *completely separate from the respiratory epithelium except at their opening into the nasal cavity anteriorly.... The vomeronasal organ has an oval lumen.... The cellular distribution of the vomeronasal epithelium is similar to that of the olfactory neuroepithelium"* (Nakashima *et al*, 1984, page 644).

The function of this structure, especially at a late stage in human foetal development, is obscure. There is also controversy surroundings its functions in the species listed above (Moulton, 1978). For this reason, it is not proposed to speculate on its appearance in human foetuses, or why it is not usually found at a later stage in human development.

Micro-anatomy.
Olfactory mucosa: ultrastructure by cell types.

1.3.1. The olfactory mucosa, which contains the olfactory receptor cells and their associated cell types, is confined to an area of about 2 cubic centimetres. There is disagreement in the literature about this figure. Some modern estimates give a much lower figure than earlier studies, which suggested an area of 10 cm^2 (e.g. von Brunn, 1892; cited in Moulton, 1978). However, even modern estimates show a considerable degree of variation. For example, Castellucci (1985) gives a figure of 5 cm^2, whereas Jenner & Dodd (1988) suggest 120 cm^2. This is the figure for the mature adult; no figures were available from the foetal study by Nakashima *et al* (1984). The olfactory mucosa contains four types of cells (Morrison & Costanzo, 1990), which will be discussed in some detail, in order to make the point that there seem to be micro-anatomical differences between infants and adults. The four cell types are:

1) microvillar cells
2) ciliated olfactory receptor cells
3) sustentacular cells
4) basal cells and the lamina propria

1.3.2. Olfactory epithelium is a pseudostriated columnar type resting on highly cellular lamina propria. The mean thickness of this epithelium is approximately 70 microns. In the human infant, the epithelium is described only as:

> *"thick and highly cellular...* [with] *a zonal distribution of supporting* [sustentacular], *sensory receptor, and basal cells"* (Morrison & Costanzo, 1990, page 643).

However, there are apparently clear differences between the adult olfactory neuroepithelium and that found in late foetuses and presumably infants. In foetuses, the epithelium has a clear zonal distribution of cells. This zoning is 'disturbed' in adults and has been attributed to ageing processes (Naessen, 1971, cited in Nakashima *et*

al, 1984). It might be speculated that this zonal disruption could be the result of long-term exposure to atmospheric pollutants. Furthermore, recent studies have shown that the olfactory epithelium in adults may contain respiratory epithelium as well, in a complex mixture (Morrison & Costanzo, 1990; Paik, Lehman, Smith & Seiden, 1990). The epithelial surface in both adults and foetuses is covered by a layer of mucous, through which odorants have to pass. The role of this mucous layer is crucial to olfaction and will be discussed later.

1.4.1. The outermost cell type encountered in the olfactory epithelium is the recently-identified **microvillar** cell. These cells are flask-shaped, with a narrow neck at the cell apex, which is equipped with short, straight microvilli that project into the mucous layer. They are usually abundant but solitary, though pairs are sometimes seen under electron microscopy. The cell population in the average human is estimated at 600 000. Their function is unknown, though they bear a resemblance to cell types known, from other species, to respond to odorants (Graziadei & Tucker, 1970; cited in Moran *et al*, 1982). Indeed, they apparently look like bipolar sensory neurons, so it may not be unreasonable to ascribe a chemoreceptive role to this cell class. However, it should be noted that not all authorities recognise this cell type as being discrete. Anholt (1989), for example, fails to mention it in his review paper, referring to only three cell types.

1.4.2. The actual sensory cells are the **ciliated olfactory receptor cells.** These cells are highly specialised, with several highly unusual features (Anholt, 1989), some of which will be addressed below. Anholt speaks of the:-

> *"remarkable plasticity and unusual morphology... several unique biochemical and electrophysiological properties".*

of olfactory receptor cells. The density of these cells is somewhat hard to estimate, because of the supposed turnover rate, but has been given as 30 000 receptors/mm^2. This gives a likely mean population of 6 000 000 ciliated olfactory receptors. However, it

should be noted that Nakashima *et al* (1984) state that it is unknown whether human olfactory receptor cells 'turn over' in the same way as has been reported in other species. Other workers have suggested different estimates of the number of olfactory receptor cells. Van Toller *et al* (1985) give an estimate of 10 000 000 olfactory receptors, though Castellucci (1985) gives a figure of ten times that amount. These varying estimates serve to highlight the problem of determining the number of olfactory receptor cells. However, the density of these cells appears to be high in the neuroepithelium, with a figure of one receptor every 3 to 5 microns.

1.4.3. The morphology of the receptor cells is reasonably well-documented from adult studies, though there is little comparative work for infants. In summary, each receptor cell is a long, slender bipolar neuron, with a dendrite sent to the surface of the epithelium and an axon sent to the olfactory bulb. Receptor cells are about 42 microns long, not including the axon. The tip of the dendrite that projects into the mucous layer has a specialised structure; a bulb-shaped modification called the olfactory vesicle. This structure is about 1.5 microns in diameter and projects some 2 microns above the epithelial surface. It is this which terminates in the olfactory cilia.

1.4.4. The cilia attached to the receptor cells are believed to greatly increase the surface area of the cells, which presumably increases the receptive area available for odour molecule transduction (Berglund & Lindvall, 1982; Anholt, 1989). Each cell has between 10 and 30 of these cilia, which are presumed to be involved in the binding of odorous molecules and hence sensory transduction (Ohno *et al*, 1981; cited in Moran *et al*, 1982; Anholt, 1989). Further evidence for the role of these cilia in olfactory transduction comes from animal work described by several workers (Berglund & Lindvall, 1982; Cagan and co-workers, cited in Moran *et al*, 1982; Dionne, 1988). However, the exact mechanism of binding, and hence transduction is still not clear in either adults or infants (Bignetti, Cattaneo, Cavaggioni, Damiani & Tirindelli, 1988).

1.4.5. As much of the work on the role of the cilia comes from animal studies, it is probably premature to generalise to humans, and particularly infants. Aside from the differences in distribution of cells between human adults and foetuses, there is no evidence for any fundamental disparity in chemoreceptive mechanisms and structures. There is histological evidence of ciliated dendrites from foetuses as young as 14 weeks post-gestational age (Chuah & Zheng, 1987). The implication from this and the other studies is that the infant olfactory neuroepithelium is morphologically similar to the adult type, but more sensitive to odorants. It could be speculated that this is due to its comparatively 'pristine' state, in that infant epithelium is both highly plastic and has not yet been exposed to the destructive effects of airborne pollutants.

1.4.6. A further distinctive property of olfactory receptor cells is that of a unique cytoplasmic protein called 'olfactory marker protein', or OMP (Margolis, 1972, cited in Nakashima *et al,* 1985; Anholt, 1989). It was originally isolated in rodents, but has also been found in humans, both adult and in the later foetal stage (Nakashima *et al,* 1985; Chuah & Zheng, 1987). It is found throughout the olfactory receptor neuron and is reported to be useful in discriminating olfactory from respiratory epithelium, though its function is unknown in either infants or adults (Nakashima *et al,* 1985). Perhaps OMP has some role in dealing with the breakdown products of odorant molecules following transduction, along with cytochrome P-450, mentioned below.

1.5.1. The third cell type found in the olfactory epithelium consists of the supportive or **sustentacular cells.** These will not be discussed in detail, though Moran *et al* (1982) describe them as:*"morphologically intriguing"*. These supporting cells lie between and around the receptor cells in the epithelium, showing a 'tight junction' relationship. It may be that they perform a supportive and nutritive function analogous to neuroglial cells in the brain. However, 'gap' junctions, which would allow electrical coupling of adjacent cells, have yet to be demonstrated. The sustentacular cells do not secrete mucous, though this is not the case in the same class of cells in the respiratory epithelium. However, it is hypothesised that

the sustentacular cells may be equipped for enzyme-based degradation and axoplasmic transport of inspired odour molecules and their metabolites (Moran *et al,* 1982). This is an intriguing idea which raises the possibility that repeated exposure to cytotoxic breakdown products acquired during lifetime inhalation of odorants may lead to the disruption of the neuroepithelium seen in the adult but not the foetus. This may be a microstructural analogue of 'presbyosmia', which is the age-associated increase in odour-detection thresholds described by Van Toller *et al* (1985). However, a cellular transport mechanism is not the sole candidate for this process of odour molecule degradation, mainly because the sheer diversity of molecules makes a unitary system less likely. An intra-cellular enzyme (cytochrome P-450 mono-oxygenase) has been suggested as a candidate for this process (Van Toller *et al,* 1985). For a review of the role of this enzyme, see Jenner & Dodd (1988). It is a powerful oxidiser of organic molecules, principally found in the liver, where it performs a detoxification role. It is also found in high concentrations in the olfactory mucosa.

1.5.2. The fourth cell type is the **basal cell.** These are typical 'stem' cells that are the progenitors of the other cell types. The undifferentiated cell is small (4 to 6 microns, about the size of an erythrocyte) and located on the basement membrane, or lamina propria. This structure, which is highly cellular, is a framework of collagenous connective tissue unlike a true submucosa. It contains blood vessels, connective tissue cells, nerve bundles and Bowman's (secretory) glands. One of the main functions of the lamina propria seems to be guidance and support, especially during neurogenesis, for the afferent axons of ciliated olfactory receptor cells. It is within the lamina propria that these axons form large bundles and travel to the cribriform plate, en route to the olfactory bulb.

1.5.3. There is no evidence that this state of affairs is any different in the infant nasal cavity. It is not known, for example, whether the basal cells are more active or numerous. However, if the lamina propria acts as a medium for developing neuronal interconnections, as suggested above, then one would expect a proportionately greater number of axons to be present in the late

human foetus and young infant. This would tend to be reduced over time as connections became established and neuronal die-back occurred. This hypothesis awaits the necessary comparative ultrastructural studies.

Section 2
Physiology of the human olfactory system

1.6.1. This chapter has already provided an overview of how odorous molecules are brought as far as the mucous layer overlaying the receptor cells. This section will deal with the physiology of the human olfactory system, with special reference to what is known about the infant system. It is not proposed to deal with this highly complex subject in great depth, as this has already been accomplished in an excellent review by Anholt (1989). Like any other human sensory system, the olfactory sense is set up to collect, transduce, process and transfer incoming environmental information. This section will consider the molecular events surrounding transduction, as well as briefly considering some of the various theories of odour transduction as applied to both adults and infants.

1.6.2. In order for any transduction to occur, odour molecules must first diffuse through the mucous layer to reach the cilia of the receptor cells. It should be noted that various authorities have calculated the possible number of odorants that the human system may be sensitive to. According to Dionne (1988), there may be 100 000 different types, though Engen (1982) suggests 400 000. Although most perceived odours are complex mixtures, pure odorants exist and it is the physical features of the molecule that partly determine its ability to reach and stimulate the receptor (Van Toller *et al*, 1985, page 19).

1.6.3. One of the problems surrounding this event is encapsulated by Van Toller (*op.cit.*):

> *"The first step in the molecular sensing mechanism is the transfer of the odorant molecules from the air into the mucous layer. The chemistry and biochemistry of the mucous is largely unknown"* . (pages 22-23).

If the fine structure of the mucous is obscure, then one of the earliest steps in odour transduction is unknown. However, Van Toller *et al* go on to suggest that the mucous comprises a solution of glycoproteins (proteins with chains of sugar molecules attached to them) in an aqueous solution, resembling mucous found on other epithelia in the human body. Water-soluble odorants will dissolve readily in the mucous, as will highly volatile lipid-soluble molecules. In this way, the essentially biochemical attributes of an odorant molecule may determine its ability to reach and stimulate the olfactory receptors. Hence the physical characteristics of an odorant may contribute to its detection threshold. Furthermore, the thickness and viscosity of the mucous layer may vary both spatially and temporally in adult humans, thus exhibiting a kind of cyclical variation (Mair *et al,* 1978; cited in Engen, 1982). This may further affect the amount of odorant reaching the receptor cells. No evidence is available in the human infant for this view.

1.7.1. How and where odorants bind to the olfactory receptors is apparently still an unanswered question (Bignetti *et al,* 1988; Anholt, 1989). This is especially the case when evidence from Dionne (1988) is considered. This worker suggests that there may be no such thing as a special protein existing just to bind odorants. As Van Toller *et al* (1985) put it:

> *"The first obvious question is whether or not the stimulant molecules bind to a receptor on the outside of the nerve cell or whether they pass into the cell and bind to an intracellular receptor"* (page 23).

According to Van Toller *et al,* there may even be a group of odorants that act on the outer cellular membrane of the receptor cells and another group that traverse the membrane and act on intracellular structures. It is not proposed to address this debate in any more detail. Suffice it to say that there is still considerable debate over the nature and location of any binding sites, despite evidence to the contrary (Pelosi *et al,* 1981, Pisanelli, 1982; cited in Bignetti *et al,* 1988). At this point, the largely biochemical arena of olfactory

transduction will be replaced by a discussion of the broader area of olfaction theories. The reason for doing this is to provide a context for how infant olfactory perception may operate.

1.8.1. There are a number of theories of olfaction. A selection of the more well-known of these will be addressed. One, at least, dates back to Roman times. The poet Lucretius put it this way:

> *"You may readily recognise that those bodies which touch our bodies pleasantly are made of smooth round atoms, but contrariwise all that seems to be bitter and rough are held in connection by atoms more hooked, and are therefore wont to tear open their way to our senses and to break the texture by their intrusion"* (cited in Van Toller *et al,* 1985).

1.8.2. Several theories have extended Lucretius' idea of specific shape being responsible for perceived odour. Amoore has presented the most elaborate and closely-reasoned of these (Amoore, 1967; cited in Berglund & Lindvall, 1982). In summary, this theory states that odour molecules attach to molecular receptors by virtue of their shape. This has been called a 'lock-and-key' view, in that the configuration of the molecule is responsible for its ability to attach to the receptor site and thus induce a change in cell potential, which amounts to transduction.

1.8.3. The actual molecular events which occur as a result of binding are more difficult to describe. Studies of other sensory systems have shown that the critical events surrounding transduction are biochemically mediated, as in the visual system (Carlson, 1981). There is no theoretical reason to assume a radically different arrangement for the olfactory system (Van Toller *et al,* 1985). However, Dionne (1988) disagrees. It is apparent that the solution to this conundrum awaits detailed biochemical evidence. Some of this is given by Anholt (1989), who provides a detailed discussion of the ionic events accompanying odorant binding.

1.8.4. The theories of Amoore and others who favour the 'stereochemical' (or 'molecular shape') models, have advanced the

study of odour transduction, even though no such highly specific proteins have been found. Another theory suggests that it is the spatiotemporal distribution of odour molecules within the mucosa that determines the perceived odour, which is analogous to chromatography (Mozell, 1970, cited in Berglund & Lindvall, 1982). However, there is nothing to preclude an eclectic mixture of these models, as Berglund & Lindvall (*op.cit.*.) state.

1.8.5. Further theories include that of Wright (1954, cited in Berglund & Lindvall, 1982). This is the so-called 'vibrational hypothesis' which states that the amount of resonance of an odorant molecule determines the degree of transduction by specialised pigment molecules in the olfactory mucosa. This hypothesis has been further argued by Briggs & Duncan (1962, cited in Berglund & Lindvall, 1982), who claimed that these pigment molecules are analogous to those found in the visual photoreceptors. Another theory, which also relates to the physical characteristics of the odorant molecules is that of Laffort *et al* (1974, cited in Berglund & Lindvall, 1982). The physico-chemical properties of the odour molecules, combined with characteristics of receptor proteins, operate in conjunction to determine the receptor membrane potentials. There is apparently some empirical evidence for this hypothesis.

1.8.6. Lastly, Holley & Döving (1977, cited in Berglund & Lindvall, 1982) suggest a complex model which relies upon the existence of odorant 'acceptors', which provide a spatiotemporal patterning to encode odour quality. Recent work in the theoretical and mathematical modelling of electrophysiological events in the olfactory bulb, based on Chaos Theory (Skarda & Freeman, 1987) have moved even closer to an eclectic approach. This research represents an attempt to explain olfactory coding, discrimination and odour memory. It attracted numerous criticisms (e.g. Brown, 1987; Corner & Noest, 1987), so it is probably fair to say that Skarda & Freeman's work is not yet proven. Nevertheless, the main value of this work is that it provokes thought about how to conceive the problem of odour perception in new ways. Such research may even

help us to consider the 'chaotic' nature of human EEG in response to odour.

1.8.7. In terms of the infant olfactory system, there is no evidence to support either one, or a combination of the above theories. However, the major difference between the infant and adult systems is likely to be the highly plastic nature of the former. There is considerable evidence that post-natal neuronal interconnection is influenced by chemosensory experience, though this evidence derives from animal research, so caution is required in generalising to the human system. This aspect will be considered in the following section, which discusses the developmental aspects of the human olfactory system.

Section 3
Development of the human olfactory system

1.9.1. Much of the evidence in this section derives from embryological work, in particular that of Lemire, Loeser, Leech & Alvord (1975). It should be emphasised that relatively little is known about the embryological development of the human olfactory system. Müller & O'Rahilly (1989) succinctly put it thus:

> *"The embryology of the human brain is very poorly documented and illustrated in contrast to that of experimental animals"* (page 353).

These and other authors are usually not primarily concerned with olfactory development *per se*, but the system is often considered interesting to those studying the ontogeny of the brain and central nervous system. This is largely because of its relative accessibility in experimental animals. Furthermore, the olfactory system is usually considered to be one of the phylogenetically oldest, and can thus be considered as a precursor to neocortical development. Some anatomists have studied discrete areas of the olfactory system during development (for example; Humphrey, 1940), but not attempted an integration of the whole area. Moreover, most authors admit that not much is known about the ontogenetic development of

the human olfactory system. This is corroborated by Gottlieb (1973; cited in Schaal, 1988b) who, referring to the chemoreceptive senses, states:*"there is little or no information on their development"*. However, what is known in terms of anatomical and neurological development will be summarised. The aim of this is to provide a background for the model described in Chapter 3 and the research described in Chapter 4.

1.9.2. The first signs of differentiation in the embryological olfactory system are the olfactory placodes, which appear at about the fourth gestational week. This corresponds to stage XIII of Streeter's embryological coding system, which will be used in this section (Streeter, 1942, 1945, 1948: cited in Lemire *et al,* 1975). Olfactory placodes are described by Costanzo & Graziadei (1986) as:

> *"Paired thickenings of the cranial ectoderm .... The nervous layer of the olfactory placodes gives rise to the receptors of the sensory epithelium; the non-nervous layer of the placodes gives rise to the supporting cells and Bowman's glands"* (page 234).

The first bipolar cells resembling olfactory nerves appear in the olfactory placode by stage XVI (about 35 gestational days) and the olfactory nerve proper is defined by stage XIX (about 50 days). The olfactory bulb begins to assume its adult macro- and micro-structure by stage XXIII, or nearly 60 gestational days. Differentiation is completed by about 100 days.

1.9.3. Several other anatomical entities appear and then apparently become vestigial during this period. These include the olfactory ventricle, the accessory olfactory bulb and the vomeronasal organ. It is not known what function these 'proto-organs' subserve, or why they degenerate into a vestigial state during embryonic development. However, it is known that the anterior nares are plugged by epithelium (Schaffer, 1910, cited in Schaal, 1988a). Smith (1976) states that this occurs from about the eighth week of gestation, until the twenty-fourth week, when they reopen. No reason is suggested for this, but it may be to 'protect' the developing

olfactory receptors from amniotic chemosensory stimulation during a crucial period in neurogenesis. The proto-nasal cavity (or 'nasal pit') is evident at stages XV and XVI, but develops into a separate nasal sac by stage XVII, about 41 post-ovulatory days (Müller & O'Rahilly, 1989).

1.9.4. The development of the micro- and ultrastructure of the foetal olfactory system is less well documented. Post-natal development is even more obscure, probably because of the paucity of cadaver material alluded to at the beginning of this chapter. However, the olfactory receptor cells may be identifiable by the ninth to eleventh week of gestation (Schaal, 1988a, page 147). The cell types described in Section 2 can be demonstrated soon after the end of the first trimester of pregnancy, according to one study (Chuah & Zheng, 1987). This may allow early receptor function to be inferred, as suggested by Pyatkina (1982, cited in Schaal, 1988a), as well as Gesteland *et al* (Gesteland, Yancey & Farbman, 1982; cited in Schaal, *op.cit.*). The appearance of olfactory marker protein in older foetuses, demonstrated by Chuah & Zheng (1987), may be a biochemical indicator of receptor cell functioning. This is certainly the case in foetal rats, though it may be premature to generalise to humans. However, Shepherd (1988) states that the olfactory system is one of the most precocious systems in early embryonic life. The implications of this precocious functioning are discussed at length in Chapter 3 of this thesis.

1.9.5. In humans, connections with the olfactory bulb and hence the rest of the brain, appear somewhat later. Using olfactory marker protein as a 'tracer', it has been shown that the olfactory bulb seems to become 'wired up' at about 32 weeks (Nakashima *et al*, 1985). However, it has been stated that, in contrast with other mammals, the human olfactory epithelium seems to develop precociously:

> *"the differentiation of the human olfactory epithelium begins relatively early as compared to other mammals such as the rat in which the receptor and supporting cells can only be identified in the second half of gestation"* (Chuah & Zheng, 1987).

No great claims may yet be made for this finding, as the possibility of functioning receptors does not necessarily imply a fully functional, cortically interconnected chemosensory system. Nonetheless, it may have implications for prenatal olfactory experience in *Homo sapiens* as discussed in Chapter 3 of this thesis.

1.9.6. To summarise the ontogeny of the human olfactory system, the following table (Table 1; adapted from Schaal, 1988a) is included:

TABLE 1: Pre-natal ontogeny of nasal chemoreceptors in humans (adapted from Schaal, 1988a)

Gestational age (post-ovulatory week)	
3.5 - 5	Formation of the olfactory placode (2,4,12)
4.5 - 6	Formation of the nasal grooves (4,12)
4.5 - 7	Differentiation of the olfactory nerves (4,12)
5 - 8	Formation of the vomeronasal grooves (2,4,12)
5.5 - 13	Presence of olfactory-like cells in the vomeronasal regions (13,14)
5.8 - 8	Terminal-like vomeronasal pathways differentiated (2,4,8,12,13)
6 - 6.5	Formation of the main olfactory bulbs (2)
6.5	Formation of the accessory olfactory bulbs (8,13)
7 - 8	Characteristic structure of the main olfactory bulbs delineated (2)
7.5 - 9.5	Opthalmic & maxillary divisions of the trigeminal nerve differentiated (5,6,9,10)
9.5	Increase of the size of the mitral cells in the main olfactory bulbs (8)
11	Presence of ciliated olfactory receptors suggested to be ready for reception (1,15)
11 - 18.5	Mitral cell layer clearly delineated in the main olfactory bulbs (8)
16 - 24	Presence of epithelial nasal plugs in the external nares (16)
24 - term	Resolution of epithelial nasal plugs in the external nares (16)
32 - 35	Olfactory marker protein present in the olfactory neuroepithelium, the olfactory nerve and the bulbar glomerular layer (3)

References: 1. Arey (1930), 2. Bossy (1980), 3. Chuah & Zheng (1987), 4. Gasser (1977), 5. Gasser & Hendrickx (1969), 6. Hogg (1941), 7. Hooker (1952), 8. Humphrey (1940), 10. Humphrey (1978), 11. O'Rahilly (1967), 12 O'Rahilly *et al* (1981), 13. Pearson (1941), 14. Pearson (1942), 15. Pyatkina (1982), 16. Schaffer (1910).

1.9.7. The post-natal neurological development of the human infant olfactory system is difficult to assess directly. As Schaal (1988a) puts it:

> *"What we know about the ontogenetic interrelations between structural and functional correlates of human olfaction remains disconcertingly poor"* (page 148).

Histological studies of the postnatal anatomy are very rare indeed. Steiner (1979, cited in Schaal, 1988a) mentions occasional cases of gross CNS malformation which responded post-natally to odorants, but no micro-anatomical details are given. However, inferences from animal studies suggest a predisposition to plasticity which may well also be valid in the human. As Shepherd (1988) puts it:

> *"Its early-developing functional properties display a considerable plasticity whose effects can last well into adult life"* (page 240).

1.9.8. The mechanisms underlying this remarkable neuronal ability to adapt structurally to sensory experience are now being uncovered. For example, molecular biology has suggested that certain proteins found in cortical neurons may underpin this ability (Aoki & Siekevitz, 1988). These authors provide a clear analogy of the process of neuronal plasticity:

> *"The developing brain can be likened to a highway system that evolves with use; less travelled roads may be abandoned, popular roads broadened and new ones added where they are needed"* (page 34).

1.9.9. It is not known whether the effects of the degree of enrichment of the olfactory environment influence the degree of plasticity, in the manner of classical studies of the feline visual system. Deprivation and enrichment studies have been carried out in the rat, but for obvious ethical reasons, not in the human infant (Eckert & Schmidt, 1985; Costanzo & Graziadei, 1986). Studies of

post-natal development in rodents are numerous and tend to confirm the uniquely high degree of plasticity of the system (e.g. Wilson, Sullivan & Leon, 1987; Royet, Jourdan & Ploye, 1989a; Royet, Jourdan, Ploye & Souchier, 1989b). Indeed, Costanzo & Graziadei (1986) go so far as to state:

> *"The capacity for neurogenesis and reconnection of pathways in the olfactory system is unique in the mammalian nervous system"* (page 146).

1.9.10. It is tempting to generalise such studies to the human, but there are several problems, as outlined in the introduction to this chapter. The first of these is that the necessary morphometric, anatomical and ultrastructural data on the developing infant brain as a whole is lacking, though many general principles of neurogenesis have been propounded (Nowakowski, 1987). This view is taken by Greenough and his colleagues (Greenough, Black & Wallace, 1987):

> *"Although research has demonstrated substantial effects of experience on brain connections, we do not yet understand just how the infant's brain is specialised to organise and incorporate experience, or the ways in which an infant may program its own experience"* (page 539).

This is particularly so in the case of the human olfactory system. As explained elsewhere, much is known about this area from animal work. However, it is unlikely that the necessary *in vivo* human studies will be done to elucidate the olfactory system in infants or adults. Because of this, plasticity in the postnatal human olfactory system may only be <u>inferred</u> rather than <u>demonstrated</u>. However, as discussed in Chapter 3 of this thesis, there are reasonable grounds for assuming, and perhaps one day demonstrating, a high degree of plasticity in the human foetal olfactory system.

1.9.11. The second problem concerns what is actually meant by 'plasticity' and whether it is a unitary phenomenon at all in the human infant. It has been suggested that there are at least two types (Greenough *et al*, 1987, cited in Bertenthal & Campos, 1987).

One is called 'experience-expectant' plasticity, and relates to the organisation of the nervous system being set up to deal with sensory experiences common to all organisms. The other is called 'experience-dependent' plasticity, and appears to be the more applicable in the human case. This type assumes a high degree of flexibility in the nervous system, because certain sensory experiences will tend to appear at unpredictable times during foetal development. An example might be the arrival in the amniotic fluid of a large quantity of a particular odorous molecule. Hence the developing nervous system has to be able to incorporate some information at any time during maturation. Whether this is indeed the case in the humans is speculative at the moment. There may even be more than two types of plasticity in a highly complex organism like the human foetus, though there is no empirical evidence either way. Indeed, as Alberts (1981, cited in Schaal, 1988a) states:

> *"Little is known about the development of olfactory function as well as about the function of olfaction during development"* (page 145).

However, in Chapter 3 of this thesis, a model is described which may go some way to answering this question.

1.9.12. To summarise this chapter, whilst much is known about the anatomy, physiology and ontogeny of the mammalian olfactory system, relatively little is known about these areas in the infant human. This is probably a reflection of the concentrated interest in other sensory modalities, which have received considerable attention (Werner & Lipsitt, 1981). Inevitably, olfaction and other sensory systems have been relatively under-researched. This scientific neglect has hitherto led to a biased view of the infant sensorium; with perhaps an inappropriate weight given to the importance of vision. This bias towards other sensory modalities continues to the present day. As an example of this, in a recent review article concerned with infant capabilities (Slater, 1990), olfaction received no mention. This can only lead to an incomplete picture of the infant's total sensory experience:

> *"the omission of taste, touch and olfaction... is... an accurate reflection of the disproportionate concern which has been afforded to vision and audition in studies of infancy.... Such an imbalance would detract from a comprehension of functioning at any stages of development, but may be particularly distorting with regard to our understanding of the world of the infant"* Türkewitz (1979, cited in Schaal, 1988a).

However, with increasing interest in the pre-natal infant world, this situation may improve. If, as argued by Schaal (1988a&b) and others, chemosensory experience and learning might begin *in utero*, then it is to be hoped that greater research resources will be directed to the study of infant olfaction.

1.9.13. It is important to understand this area in order to avoid the sort of distorted picture Türkewitz described. Although it is clear that older infants are visually dominant, this may not be the case for the immediate post-natal period. It seems possible that, in the first few weeks, or even months after birth, the infant requires a communications channel to the mother. This is, of course, non-linguistic. In the period before the visual system has developed sufficiently to permit high-capacity information input to the infant, the olfactory system may function as such a communications channel. The relative post-natal precociousness and probable high degree of plasticity in this system may allow some level of information exchange within the mother-infant dyad. This argument is advanced in greater detail in Chapter 3. However, the next chapter reviews previous research in the area of 'infant olfaction', with the aim of providing the historical and epistemological context for the empirical research described in Chapter 4.

CHAPTER 2
A REVIEW OF THE LITERATURE IN THE FIELD OF 'INFANT OLFACTION'

Introduction

2.1.0. The aim of this chapter is to describe and evaluate the previous scientific research in infant olfaction. The purpose of this is twofold. Firstly, to chart progress in the field and identify key contributors to the field. The second purpose is to provide a context for the studies described in Chapter 4. The broad strands of the research literature will be examined, as far as possible in chronological or related order and an attempt made to integrate the findings. The pertinent literature is broadly summarised in Table 2. The aim of this Table is to suggest groupings whereby the inter-relationships of the literature body can be made clearer and therefore this chapter will have sub-headings which refer to Table 2. It should be emphasised that the groupings are not mutually exclusive and that, for reasons of clarity, not all the studies discussed in this chapter are included in the Table. This especially refers to clinical papers.

2.1.1. The main conclusions of this chapter are that definite groupings in the literature are identifiable, despite the relatively small number of papers in this area. The relatively meagre size of the literature *corpus* in human infant olfaction, compared with the rest of the published work in infant psychology, may best be considered as due to the lack of interest in this subject by Science. This is rather than any inherent lack of importance of infant olfaction in the overall study of infant psychobiology. Indeed, as argued in Chapter 3 of this thesis, olfaction may be amongst the first sensory modalities to become functional in the infant. This is the view implied by some of the work considered in the following chapter.

TABLE 2 - A BROAD SUMMARY OF GROUPINGS IN 'INFANT OLFACTION' LITERATURE 1699-1990

GROUP 1 - EARLY OLFACTION OBSERVERS

Locke (1699); Kussmaul (1859); Genzmer (1873); Darwin (1877); Preyer (1881); Kroner (1882); Shinn (1894); Garbini (1896); Tanner (1904); Krasnogorski (1913).

GROUP 2 - TWENTIETH CENTURY PSYCHOPHYSICAL STUDIES (BEFORE WORLD WAR II)

Peterson & Rainey (1910); Canestrini (1913); Peiper (1924); Pratt, Nelson & Sun (1930); Disher (1934); Ciurlo (1934); Stirnimann (1936).

GROUP 3 - TWENTIETH CENTURY PSYCHOPHYSICAL STUDIES (AFTER WORLD WAR II)

Bronshtein *et al* (1958); Engen, Lipsitt & Kaye (1963); Engen & Lipsitt (1965); Engen, Cain & Rovee (1968); Rovee (1969, 1972); Engen & Corbit (1970); Murray & Campbell (1970); Engen & Moskowitz (1972); Self, Horowitz & Paden (1972); Rieser, Yonas & Wikner (1976); Shimada. *et al* (1986); Engen (1986, 1988); Kendal-Reed *et al* (1986, 1989, 1990); Lott, Sullivan, & McPherson, (1989); Schmidt *et al* (1989, 1990).

GROUP 4 - TWENTIETH CENTURY 'FREUDIANS'

Stein, Ottenberg & Roulet (1958); Peto (1973).

GROUP 5 - 'FACIAL EXPRESSION' STUDIES

Steiner *et al* (1973, 1975, 1977, 1979, 1990); Kendal-Reed *et al* (1986).

GROUP 6 - 'SOCIAL AND ETHOLOGICAL OLFACTION' STUDIES

Peto (1935); Macfarlane (1975); Russell (1976); Doty (1981, 1986); Porter *et al* (1981, 1983, 1985, 1986, 1988, 1989); Filsinger & Fabes (1985, 1986, 1988); Schaal *et al* (1980, 1984a&b, 1986, 1987, 1988a&b).

GROUP 7 - CLINICAL STUDIES

Sarnat (1978); Datta *et al* (1982); Gauthaman *et al* (1984).

2.1.2. Several of the studies shown in Table 2 are argued to be seminal in the area of infant olfaction and are therefore discussed in detail. The remainder are summarised, and any unusual or important features examined more closely. It should be made clear that neither animal studies, nor general review papers are discussed (e.g. Lawless, 1985; Mykytowycz; 1985; or Haith, 1986). These latter papers serve to collect together parts of the literature for a general scientific readership and tend not to add any new thought or research. A few studies concerned with olfactory perception in older children are, however included where they are also relevant to infant olfaction. For the sake of completeness, it should be noted that a few exclusively clinical studies have been published (Sarnat, 1978; Datta, Prasad & George, 1982; Gauthaman, Jayachandran, & Prabhaka, 1984). Such studies are placed together as Group 7 in Table 2. These papers are concerned only with using the olfactory system to test the integrity of the neonatal central nervous system for diagnostic and prognostic purposes. The studies will not be discussed as they are tangential to the aims of this chapter. Table 3 contains a glossary of the odours and chemicals mentioned in this thesis.

The Early Olfaction Observers (Group 1, Table 2)

2.2.1. Historically, there have only been a few published accounts of infant odour perception. Nonetheless, some early, mainly anecdotal accounts of the role of odour in infancy do exist. One of the earliest is that of John Locke, the philosopher, writing in 1699 (cited in Kessen, 1965). Locke suggested that odour could be used to exert a therapeutic effect:

> *"If the newborn baby is in a weak condition you can blow on it the smell of chewed onions and cloves; smear its nostrils and lips with Cinnamon water"* (pages 58-59).

Even Charles Darwin felt inclined to comment in this area. In his 1877 essay **"A Biographical Sketch of an Infant"** he discussed the factors mediating suckling (cited in Kessen, 1965). However, he confessed to being unsure about the role of olfaction in the behaviour of his own son.

TABLE 3 - A GLOSSARY OF ODOURS AND CHEMICALS

Note that some of the odours given in this table are their own descriptors, and no better descriptive label exists.

Alcohol, ethyl. Ethanol [C_2H_5OH]. Used as a diluent in perfume manufacture. 'Sharp' odour that is often a top-note constituent until it evaporates.

Alcohol, phenylethyl. [$CH_2.CH_2.OH$]. Gives a 'rose' note, but possibly a green, 'hyacinth' note if contaminated by aldehydes.

Alcohols, aliphatic. A series comprising propanol, pentanol, hexanol, octanol and decanol. Give a vaguely 'oily' or even slightly 'fatty' note.

Amber, oil of. An ambiguous odorant. Possibly refers to 'ambergris' (a costly perfume ingredient) giving a 'balsamic' note.

Ammonia. A mainly trigeminal stimulant - very 'sharp' and pungent.

Ammonium hydroxide. An ammoniacal odour, as above.

Androstenone. 5-alpha-andros-16-en-3-one. An odorous steroid found in human sweat, particularly in males. To those able to smell it, it has a 'urinous' or even 'musky' odour. About half the population have a specific anosmia to this chemical.

Anise. A liquorice odour.

Asafoetida. Otherwise known by its common name of 'Devil's Dung'. An unpleasant, 'foul' odour, depending on concentration.

Banana. A 'fruity' odour.

Bromine. A toxic halogen. A 'suffocating', or 'choking' odour, not unlike ammonia (q.v.).

Camphor. A camphoraceous, 'mothball'-like odour.

Chenopodium, oil of. An ambiguous term. Possibly an unpleasant odour reminiscent of Syrup of Figs.

Clove oil. A 'spicy', slightly 'floral' odour.

Gentian, tincture of. Often used as a dye or wound dressing. Should be odourless, unless diluted by alcohol.

Geranium, rose oil of. A roseaceous, 'floral' odour.

Hydrogen sulphide. H_2S. A putrid odour, normally attributed to 'rotten eggs'. One of the few 'pure' olfactory odorants.

Iodoform. An irritant odour, related to the halogen iodine. Toxic.

Lavender. An 'herbaceous', 'floral' odour.

Mint. A 'minty' odour, possibly menthone.

Mother's milk. Very complex constituents. Several candidates for the odour produced by this substance, including isobutyraldehyde. This gives a vaguely 'malty' odour.

Orange, compound spirits of. An ambiguous term. Possibly an orange-like, 'fruity' odour.

Peppermint. See mint.

Petroleum. Constituents very variable prior to modern refining processes. Possibly a 'sulphorous', 'oily' odour.

Polyethylene glycol. ($H[OCH_2CH_2]_4OH$). A filling and emulsifying agent used in the food manufacturing industry. Harmless and virtually odourless in use. Often employed in odour experiments as a diluent agent or vehicle.

Shrimp, artificial odour of. Probably the 'fishy' note of trimethylamine $(CH_3)_3N$.

Skin odour. Extremely complex constituents, consisting of odorous steroids (see androstenone, above) and fatty acids, amongst many others. One constituent might be the 'sweaty' odour of isovaleric acid (see valerian, below).

Succinic acid. $CO_2H(CH_2)_2CO_2H$. Used as a buffer, and in the manufacture of lacquers and dyes. In the pure state, this chemical should be odourless. Odour probably due to impurities.

Valerian. Variously described as a 'goaty', 'sweaty' or 'animalic' note.

Vinegar. A dilute form of acetic acid (CH_3OOH), with a sharp, 'vinegary' note. A trigeminal stimulant.

2.2.2. In terms of experimental research, as opposed to anecdotal studies, credit must go to Kussmaul (1859) for the first published account of infant olfaction experiments. Kussmaul tested 21 infants with asafoetida, acetic acid, and even ammonia. These odours, and others mentioned in this and other chapters, are described in more detail in the Glossary (Table 3). All Kussmaul's subjects were asleep at the time of testing. General motor reactions were seen and the infant subjects awoke when presented with the odours. Even premature infants reacted strongly to unpleasant stimuli in the first day of life. Furthermore, a response decrement was seen on retesting, which may be one of the earliest accounts of habituation to odour. The phenomenon of habituation to repetitive stimulation is, of course, a common one in psychology and its importance in olfaction will be discussed in greater length later in this thesis. Kussmaul did not offer an explanation for the response decrement, but habituation to odours, especially aversive ones, would have been recognised well before the mid 19th. century.

2.2.3. Kussmaul also mentioned the trigeminal ('tactile') aspects of odour perception, so he was clearly aware of the difficulty of separating the trigeminal and olfactory components in odour perception. This is remarkable because some subsequent researchers have seemed either to be unaware of this, or rather to have ignored it. This topic has recently been addressed most eloquently in adult studies by Kobal and his colleagues (Kobal & Hummel, 1988; Kobal & Hummel, 1989; Hummel & Kobal, 1990).

2.2.4. Most of Kussmaul's stimuli were what adults would term 'aversive' or unpleasant. Subsequent studies have shown that such value judgements are probably learned (Peto, 1935). As yet, no odorant has been found which is inherently aversive to children and infants (Engen & Corbit, 1970; Engen & Moskowitz, 1972). Kussmaul's use of sleeping subjects is not unusual. However, as Murray & Campbell (1970) point out, the various degrees of 'state' in terms of wakefulness, can have a profound difference on olfactory threshold.

2.3.1. Genzmer[1] carried out similar work in 1873 on twenty neonates. However, his paradigm differed in that only one stimulus was used, which was asafoetida. Genzmer's conclusions were broadly similar to those of Kussmaul, except that Genzmer asserts that infant recognition of the maternal breast by odour is not possible in the first eight days of life. However, no empirical evidence for this assertion was offered. Modern studies have demonstrated that infant recognition of maternal odour may indeed occur (Stein, Ottenberg & Roulet, 1958; Russell, 1976; Schaal, Montagner, Hertling, Bolzoni & Moyse, 1980). Genzmer's work is interesting in that the odour stimulus was applied to the subjects' upper lip rather than being presented to the anterior nares. Clearly, the tactile component of the stimulus would tend to obscure any response to such odorants. Perhaps he was hoping to standardise or even prolong the odour presentation and felt this was the best method. In general, Genzmer's study appears less rigorous than Kussmaul's work.

2.3.2. The work of Kroner (1882)[2], appears to be a natural progression to that of Genzmer's, though there is no evidence of prior knowledge of this work. They were working in a similar *Zeitgeist* and followed similar paths. However, in contrast to Genzmer, Kroner was able to elicit reactions to odorants within 15 minutes of birth; he also noted a response decrement over successive trials. Whether this reflects a difference in technique, or more precise observation is not known. Kroner was apparently interested in what factors initiated suckling. Probably his hypothesis was that maternal odour, or perhaps that of breast milk was involved. In any case, he tried the effect of smearing oil of amber or petroleum on the breast of a lactating woman. Not surprisingly, her baby refused to suckle from this breast. It would seem likely that it was the trigeminal component of petrol vapour, or the oil of amber that caused this. It would seem an odd choice of odorants on Kroner's behalf, but perhaps he was merely looking for a very obvious effect produced by powerful odours. He apparently concluded that babies recognise their own mother's breast by touch.

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[1] cited in Disher (1934)
[2] cited in Disher (1934)

2.4.1. Preyer (1881)[3] published work similar to that of Kroner. Preyer stated that his subjects refused a breast with the disagreeable odour; also that refusal of cow's milk after initial breastfeeding was due to odour, not taste. He also asserted that location of the nipple is unlikely to be mediated by odour and that olfaction is not of great importance to infants. However, Preyer clearly believed that stimulation of subjects by ammonia and acetic acid vapour acts as an irritant to the nasal mucous membrane. Reactions of his subjects were listed as: violent sneezing, corrugation of forehead or blinking and rubbing of face with hands. Asafoetida elicited crying and sucking reactions.

2.4.2. Preyer's work seems more anecdotal than both earlier workers and his German contemporaries. However, as Pratt, Nelson & Sun (1930) comment, he secured the same reactions to stimuli as did other investigators. However, not all investigators were obtaining similar results and some claimed no neonatal odour reaction. Examples of this latter group are Tanner (1904)[4]; Pratt *et al*, 1930; Shinn (1894) and Garbini (1896)[5]. For example, Shinn stated that an infant living "under normal conditions" shows no reaction to smell stimuli during the first 10 months of life. Garbini went even further than this, stating categorically that there is no olfactory function until the age of 14 months.

2.4.3. Other workers claimed some reactions. Examples of this group are Kussmaul, Genzmer, and Peterson & Rainey (1910). Krasnogorski (1913) mentioned, in passing, that seven and eight month old children can discriminate the smells of perfume and camphor. This dichotomy of views is presumably due to either different operational definitions, or differences in observational rigour. This particular problem of response interpretation is not, of course, confined to late nineteenth century observational studies. With experimental psychology in its infancy, wide variations in inter-rater reliability and problems of response interpretation might

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[3] cited in Pratt *et al* (1930)
[4] cited in Pratt *et al* (1930)
[5] Shinn and Garbini both cited in Disher (1934)

have been even more of a problem than it currently is. This topic is discussed at length elsewhere in this thesis.

2.4.4. Garbini, working around the close of the nineteenth century, was of the view that olfaction is not important to the human baby until several months after birth. His work is of interest because it reiterates the distinctions made by Kussmaul between olfactory and trigeminal stimuli. He studied neonates and was unable to elicit any reaction to olfactory stimuli in the first three hours postpartum. Garbini concluded that there is total anosmia at this time. He employed acetic acid, ammonia, mustard and tobacco, naming these stimuli "osmo-tactile". This is presumably a reference to the likely chemosensory effect of these odorants. He also used petroleum, succinic acid, asafoetida, iodine and bromine. These odorants produced slight "withdrawal reactions". Using what he called "purely olfactory stimuli" (e.g. wild bedbugs and putrefying meat!) Garbini noted slight "repellent movements". All stimuli were presented as the subjects took the breast. Presumably, because his main conclusion was that olfactory sensations do not appear until the fourteenth month of life, his operational definitions of 'response' were different from other workers. Observer bias might also have been a problem. This is especially likely because the need for peer review and independent corroboration of results had not yet fully developed as part of the scientific method. A further explanation might be that such complex odours would not be 'biologically significant' to babies of this age. This concept is discussed in Chapter 3.

2.5.1. By the end of the nineteenth century, it is possible to identify two dichotomous schools of thought. The first of these schools comprised those who seemed to claim no olfactory response in infants. The other identifiable school consisted of those who found some reactions to odorants. However, as will be addressed below, the early part of the twentieth century saw the publication of several key studies in favour of the latter school. Nonetheless, even as late as 1921, we have Drummond[6] asserting that neonates probably have no sense of smell at all. She further suggested that the reactions

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[6] cited in Disher (1934)

elicited from odorants are similar to those from tastants and that the two senses only differentiate slowly. However, Drummond's work does not appear to have involved any experimental studies. It is possible that Drummond was alluding to the problem of the significance of odours to very young infants. Many of the studies described above used highly complex odours that might not be expected to elicit reliable reactions from this age group. However, despite this problem a number of well-conducted studies obtained important empirical data in the early twentieth century.

Twentieth Century Psychophysical Studies Prior to World War II (Group 2, Table 2)

2.6.1. The first of these was that of Peterson & Rainey (1910). This was probably the first large scale scientific study of general infant perception, with over 1 000 babies tested. Peterson & Rainey used five olfactory stimuli; asafoetida, compound spirits of orange, oil of rose geranium, tincture of gentian and mother's milk. These workers tested 48 of their subject pool in the first six hours of life using the dependent measures of facial responses, sucking and crying behaviour and general motor reactions. The general conclusion was that all subjects could react shortly after birth to the odorants and this was stated in the following way:

> *"In the tests of smell in 48 infants during the first six hours after birth, we think the evidence conclusive that the olfactory nerve is sensitive to odours practically at birth"* (page 114).

2.6.2. Some twins and also some premature infants were tested. For these subjects, very similar conclusions were drawn:

> *"The olfactory nerve is ready to receive smell impressions sometime before the end of the normal period of gestation"* (page 121).

The importance of this latter conclusion is that it coincides with scientific advances in embryology and sensory physiology, which tend to corroborate the purely psychophysical conclusions. Previous investigators would not have had the extensive advantage of

knowledge gleaned from more advanced microscopy, histology and electrophysiology. Furthermore, this study was amongst the first to show clear evidence of neonatal olfactory perception. Peterson & Rainey made some attempt to control for extraneous variables, though in terms of modern psychological experiments their work was not corroborated by statistical evaluation of the data. However, this work can be seen as a junction between earlier, observational studies and later, more scientifically rigorous work.

2.7.1. Another important study in the same tradition was the work of Canestrini (1913), published in Germany. This worker reported a number of extensive and careful experiments. In particular, Canestrini clearly took pains to avoid any direct tactile stimulation and thus precluded a major, if obvious confounding variable. Canestrini was probably amongst the first in this field to use direct physical measurement. He used polygraphic techniques to measure respiration and fontanelle pulsations as indices of autonomic change, and produced tracings that look remarkably similar to those deriving from modern polygraphs.

2.7.2. Canestrini used soaked cotton applicators or sniff bottles as experimental stimuli. This would have introduced some degree of stimulus standardisation, in terms of saturation and 'head-space' of odorants. This means that if the cotton applicators were saturated with the odorant, then the concentration of odour reaching the subject would be at its maximum. However, the purity of the odorant would probably have been highly variable. This would have been a problem common to nearly all these early studies. In terms of 'head-space', if all the sniff bottles were of the same shape and internal volume, then the amount of vaporised odour above the chemical compound would have tended to be similar. This would mean that that the concentration of odour would stabilise at a given concentration. However, both these considerations are clearly dependent on many factors.

2.7.3. Canestrini's main conclusions can be summarised as follows;

1) Subjects react most strongly to those odorants which stimulate the "trigeminal components of the sense of smell".
2) Olfaction is the least active of the sensory systems in the neonate.
3) Those stimuli which do elicit a reaction cause changes in fontanelle pulsation and respiration rate/type.
4) No positive results were obtained from the use of cow's milk as a stimulus.
5) 'Aversive' stimuli sometimes act to quiet the subjects.

2.7.4. These findings are of interest, as they represent the first published psychophysical measurements in infant olfactory psychology. This study represents a major body of evidence in favour of the 'olfactory infant' school. This is the case even though Canestrini concludes that the olfactory sense is relatively inactive in infants. This probably derives, in this study as in previous ones, from the wide between-subject variation in response to odour. Such variation could derive from a large number of factors, not least the differences in the state (in terms of awake/asleep and gradations of these states) between subjects at the time of testing. This consideration has been shown to have an effect on apparent olfactory threshold in neonates (Murray & Campbell, 1970). However, those subjects who did react to the stimuli demonstrated physiological changes evidenced by autonomic responses.

2.7.5. Also of interest is Canestrini's finding that 'aversive' stimuli produced the opposite effect from that which might be expected. This calls into question the whole area of what constitutes an 'hedonic' or 'aversive' stimulus. Intuitively, this might be defined in terms of the reaction produced. Hedonic odorants have been used for many centuries for their calming effects and there is some evidence that certain odorants produce physiological effects consistent with 'bodily relaxation' (King, 1988). Generally speaking, odours rated 'pleasant' by adults, consistently produce a pleasurable reaction in a sample of subjects from the same cultural milieu. The

opposite tends to be true of adults faced with unpleasant odours. This value judgement seems to be due to received cultural knowledge producing a fairly consistent behavioural repertoire. This includes stereotypical behavioural manifestations of disgust, dislike and rejection produced when these odours are encountered.

2.7.6. There has been little previous evidence that infants and small children produce the same olfactory reactions as adults, or even older children to odours (Peto, 1935; Stein *et al*, 1958; Engen & Corbit, 1970). In other words, an odour deemed 'aversive' by an adult and producing a socially appropriate response in most people, may produce no response or even an 'hedonic' response in an infant or young child. However, Schmidt & Beauchamp (1987, 1988) reported that three-year olds display odour likes and dislikes that are similar to adult preferences. Schmidt (1990) also reported similar findings with nine month-old infants. Canestrini may not have been the first to notice this, but he seems to have been the first to publish this finding. His study is hence a milestone in the history of infant olfactory psychology. This work clearly stimulated some replication studies, such as that of Peiper (1924; cited in Pratt, Nelson & Sun (1930). This was somewhat similar to the studies of Canestrini and concluded that the sense of smell is the most difficult to stimulate of all the senses. In this context, he presumably meant 'in relative terms'.

2.8.1. The next major study in the empirical tradition is that of Pratt, Nelson & Sun (1930), which was a large psychophysical study somewhat in the style of Canestrini. The authors seemed intent on controlling as many extraneous variables as possible. They employed an elaborate, mobile experimental chamber, containing apparatus for testing various sensory modalities. They also constructed an olfactometer, which is a device for controlling temperature, saturation and humidity of odorants. Such an apparatus was, incidentally, first described in about 1850 by Valentin (Wenzel, 1948). The version used by Pratt and his colleagues directed a known volume of odorant into the infants' nostrils. In practice, it is unlikely that was delivered this precisely, though it showed an awareness of the need to standardise both

stimuli and stimulus delivery. The observational techniques were also rigidly operationalised. Two of the stimuli that were used (acetic acid and ammonia) were trigeminal stimulants rather than purely olfactory ones. This may account for the more vigorous reactions that were observed.

2.8.2. Another interesting aspect of this study is their use of stabilimetry. This technique, which measures general motor reactions, is a form of accelerometry. The stabilimeter measured the infants' movement in two planes, which was recorded on a polygraph. Odorous stimuli were delivered by means of a basic olfactometer. The use of such elaborate apparatus demonstrates a concern with control of extraneous variables to ensure, as far as possible, a standardised technique.

2.8.3. The authors tested 48 subjects in this series. The bulk of their report is in the form of summary tables, detailing several aspects of the data as well as percentage reactions elicited by the stimuli. The whole study is concisely summarised in Disher (1934). The main conclusion of the study is succinct:

> *"The results on smell stimuli confirm the conclusions from light and sound reactions, namely, that the newborn infant does not react to stimuli in the same way as do adults"* (page 143).

From the very large amount of data produced by this study, this appears to be a cautious conclusion. Furthermore, there seems to be very little theoretical basis to this work. Perhaps the authors were content merely to map infant sensory reactions across all sensory modalities without feeling the need to work from a hypothesis. Nonetheless, it is another important piece of work, because it continued to establish a major strand of research in the area of infant olfactory psychophysics. This area was further explored four years later by Disher, working in the same research environment.

2.9.1. Disher (1934) performed a number of psychological studies controlling for as many variables as possible, and over 90 infants were tested. In her introduction she highlighted some of the fundamental problems of infant olfactory psychology:

> *"Whether or not the newborn infant can smell can be determined only by an extended investigation that will cover a number of years. This is especially true because of our indefinite knowledge concerning the olfactory stimulus, the difficulty of obtaining olfactory stimuli that are not also trigeminal stimuli, and the enormous variability in reactions from infant to infant"* (page 1).

It is salutary to consider that the research of the fifty or so years since that statement seems to have done little to invalidate it. It would seem unlikely that the difficulty of between-subject variability will be overcome, as it is probably governed by factors over which psychologists have little or no control. However, Disher made a very good start in controlling for those extraneous variables that can be identified. Not only that, but the first part of her paper contains an excellent review of the literature.

2.9.2. Disher employed some elaborate apparatus in her infant work. Part of this was an olfactometer, derived from the design used by Pratt and his colleagues, to ensure filtration and saturation of the odorants. Disher's use of improved olfactometry probably derives from criticisms made in the first part of her paper of Pratt's earlier study. For this reason, Disher did not use the stabilimetry apparatus, claiming that it distorted rather than elucidated data. Eight stimuli were used representing 'corners' of Henning's odour pyramid (Henning, 1916) and "pure air" as a control. Disher clearly recognised the limitations of this classification system, which was later discredited, but probably used it because it was the sole system with any empirical support at that time.

2.9.3. Dependent measures in this study were as follows; general motor responses, vocalisations, type of respiration and facial

expressions. The latter responses were corroborated by a movie record. The conclusions were that the infants were able to react to all the stimuli and, in common with most other studies, wide between-subject response variability was seen. However, the conclusion from this study is somewhat conservative, primarily because of uncertainty over the differences between olfactory and chemosensory stimulation. Disher concluded, somewhat concisely, that very little could said about infant olfactory response. The importance of this study, however, needs emphasising. It was, of course, directly related to the work of Pratt *et al* (1930), but an improvement upon this earlier work. The main improvement was in the care taken with the olfactometry. Doubtless not all of the technical problems were solved, but a major source of variability was addressed.

2.9.4. The methodology of this and Pratt's study has had substantial influence on later work (e.g. Steiner, 1979; Engen *et al* 1963, 1965). It is probably fair to say that these two studies are the key ones of the pre-World War II period. With their careful considerations of method and attention to detail, they serve as paradigms. This is not solely a result of technical improvements or increased resources. It is rather that the thinking behind them has moved on from the view that the olfactory sense is somehow irrelevant in infants. Both these works imply that the olfactory sense is a modality that can be measured in young infants just like any other. This conceptual basis fuels most subsequent work in this area.

2.10.1. Two further studies in the 1930's corroborate this. These are Ciurlo (1934) and Stirnimann (1936). The first of these was a study using respiratory plethysmography to assess the reactions of neonates to odours. This technique involved measurement of chest wall excursion by means of a belt worn on the chest or abdomen and connected to some form of transducer. This is, in turn, was connected to a polygraph. Stimuli were apparently chosen as non-trigeminal stimulants; orange, lavender, valerian and mint. Subjects showed definite changes in respiratory amplitude and rate in response to the stimuli. The use of plethysmography echoes

the work of Canestrini in its attempt to quantify autonomic changes and is a further example of the 'psychophysical' school. This same plethysmographic technique was subsequently used by Murray & Campbell (1970) and Kendal-Reed, Kaplan & Werner (1986).

2.10.2. The work of Stirnimann (1936) is similar to that of Disher. Stirnimann tested 100 newborns in the first day of life, before their first feed, with anise, oil of chenopodium and ammonia. Generalised motor responses were measured, though many of the reactions were ambiguous and reversed on retesting. He stated that stimulation of the olfactory nerve is more easily achieved than that of the trigeminal, though little evidence is offered for this assertion. Stirnimann concluded that the infants could differentiate between the odours he used. This final point is important because it appears to relate to the function of the olfactory sense in infants. Stirnimann was clearly moving towards a sense of 'ecological validity', in that he was concerned about the everyday meaning of olfaction to the neonate. His work implies that thinking was moving on from the question "Can infants smell at all?", to considering the problem of "What can infants do with their sense of smell and what does it mean to them?".

2.11.1. It would therefore seem that the first third of the twentieth century saw the rise of the experimental approach to infant olfaction. The progressive development of experimental technique and more precise methodologies was essentially incremental, as in most sciences. The studies in this tradition would have been driven by the general hypothesis that infants have at least some olfactory capabilities and that more precise methods were needed to elicit the scope of these. The development of better polygraphic techniques assisted this epistemology. The concept of 'naturalistic' investigation did not appear in most of these studies, for the clear reason that the experimenters were trying to control for as many extraneous variables as possible to maximise experimental power. However, this was not true of all studies in this era.

2.11.2. One quite different study, which was contemporary with most of the above work, is that of Peto (1935). This work

stands out from others because of its naturalistic approach and essentially anthropological basis. Peto tested over 200 children using sniff-bottles containing stimuli representative of Henning's classification. This study assumed that infant and child subjects had adult-like thresholds, and that pre-linguistic children have a definable olfactory sense. Peto excluded odours with a predominantly trigeminal effect. Subjects were divided into three groups: under 5, 5-6 years and over 6. Younger subjects showed no reaction to "disagreeable" stimuli; the middle group were equivocal. The older group displayed more adult-like 'aversive' reactions. For those younger subjects classed as neonates or infants, few reactions, limited to limb movements and sucking movements were recorded. Peto disclaimed the view that this was due simply to immaturity of the olfactory sense. He stated that aversive type reactions to unpleasant odours are due solely to cultural expectations. The tenor of his concluding remarks suggests an anthropological, or even psychoanalytical explanation, though this latter view is not put explicitly.

2.11.3. This work was unique in its time. The departure from the prevalent experimental methodology in favour of what would nowadays be called 'ecological validity' is interesting. The stated reasons for this are both humane and pragmatic:

> *"We did not use any of the various olfactometers, we were not concerned with quantitative results. Besides the children would have been afraid of the complicated instruments"* (page 315).

2.11.4. It would seem that Peto's principal concern was for a naturalistic environment which would elicit everyday, meaningful reactions to odorants. In terms of the infants tested, very few reactions were obtained even with supra-threshold odour strengths. This was interpreted that the hedonic/aversive dimension is inapplicable in this age-group. This, as Peto put it, is due to the fact that infants have yet to learn which smells are aversive and which pleasant. It appears that Peto was the first infant olfaction researcher to make this important point. Studies prior to this had

always presupposed either a somewhat immature olfactory system in infants, or infants equipped with adult-like perception.

2.11.5. Peto stated that neither is the whole story. He claimed that there was a time of transition at about six years of age. This transition was between the infant's general acceptance of, or indifference to odours and the cultural consensus displayed by adults. The reasons for this are probably due to the intensive cultural education, which was previously begun within the family, and later extended as the child enters school and a wider social network. The process of learning and internalising cultural norms, including those of reactions to odours, would presumably tend to accelerate within such a network.

2.11.6. A further important point of Peto's concerns the importance of smell to the infant. He put it thus:

> *"It is demonstrably incorrect to speak of the sense of smell in children as underdeveloped. It is certain that in the first weeks of life, of all the senses, smell, taste (and to a certain degree touch) play the important roles: sight and hearing acquire importance only in the following months"* (page 319).

This was, and is, a contentious point in a paper full of interesting and advanced notions. Essentially, Peto is stating that the infant human is inherently responsive to odour, which goes against some of the received wisdom even today. Such a view must have seemed yet more radical, even heretical, when it was made. This is because it went against the burgeoning school of Behaviourism, which dominated psychology at the time in the USA. The possibility of innate odour responsiveness is discussed in Chapter 3 of this thesis.

2.11.7. To summarise the importance of Peto in the literature, he provided an alternative view of infant olfaction. His emphasis on the naturalistic importance of odour to the infant may be seen as the progenitor of much important work in what could be termed 'social olfaction'. This area is discussed later in this chapter. Peto stands out for his departure from the rigidly empirical thinking of most of

his contemporaries. As a footnote, it should be mentioned that Peto went on to become a renowned psychoanalyst in the USA, retaining his interest in the development of olfaction (Peto, 1973).

Twentieth Century 'Freudians' (Group 4, Table 2)

2.12.1. Following the scientific disruption during World War Two, infant olfactory research restarted in the 1950's. Most of these studies are recognisably influenced by the pre-war empirical studies described above. This might be have been due to the pervasive influence of Behaviourism, with its concern for stimulus-response links elucidated by tightly-controlled experiments. However, this was not always the case. For example, Stein, Ottenberg & Roulet (1958) conducted a study involving young children rather than infants and found similar results to Peto (1935). They began their paper by stating that the sense of smell is present at birth and important in mother-infant communication. However, they offered little in the way of evidence for this. Their findings were interpreted in classic Freudian terms relating to the Oedipal conflict, which would have presumably been anathema to the Behaviourist viewpoint.

Twentieth Century Psychophysical Studies After World War II (Group 3, Table 2)

2.12.2. A post-war study that did reflect the empirical tradition in infant olfaction came from the Soviet Union (Bronshtein, Antonova, Kamenetskaya, Lupova & Sytova,1958). This was an essentially biological dissertation on all the sensory modalities. For the olfactory sense, reactions to anise, peppermint and iodoform were recorded polygraphically. Over half of the subjects apparently gave a distinct reaction to these odorants, though few details of experimental technique were given. Nevertheless, the authors concluded that neonates and infants under one month can distinguish differences between odours. The main finding of interest, which receives little more than a passing mention in this work, was that of response decrement. This had been noted in earlier studies, for example, that of Kroner. This is presumably due to the phenomenon of habituation, an area of interest to learning theorists (e.g. Sokolov, 1963; Thompson & Spencer, 1966; Groves & Thompson, 1970) and investigated a few years later by Engen & Lipsitt (1965).

2.12.3. A small study, not dissimilar to Bronshtein *et al* was recently reported by Lott, Sullivan, & McPherson, (1989). These workers conditioned neonates to "a novel odor" (unspecified) and noted the presence of associative learning, which may form the basis of habituation. However, this recent study and that of Bronshtein *et al* are really only of passing interest, except that they mention habituation in an olfactory context. The early 1960's brought the first studies in an important series of experiments conducted by the doyen of olfactory psychologists, Trygg Engen.

2.13.1. These studies were in the classic American experimental tradition. The first experiment (Engen, Lipsitt & Kaye, 1963) followed almost directly from the work of Disher (1934). Normal neonates were tested by means of stabilimetry (q.v.) and polygraph, using both 'hedonic' and 'aversive' stimuli. The odours used were acetic acid, asafoetida, phenylethyl alcohol and anise oil. No differences in response between these odorant groups were noted, though some order effects were seen and both adaptation and response recovery were observed. This aspect of infant olfaction was further addressed in a study two years later, but in the meantime, the same team of researchers looked at changes in olfactory threshold.

2.13.2. This study (Lipsitt, Engen & Kaye, 1963) was a within-subjects design testing neonates in their first four days, using various solution strengths of asafoetida to establish response thresholds. They employed the same dependent measures as in their earlier work. Results pointed to a decrease in olfactory threshold over time, which corroborated data from other sensory modalities, though there was little theoretical discussion of this finding. Engen & Lipsitt (1965) then went on to consider response decrement in neonates.

2.13.3. In this study, 70 subjects were tested using what had become the standard Brown University techniques, as described above. Results showed evidence for habituation being the cause of response decrement rather than sensory cell fatigue. A discussion of the mechanisms underlying habituation is outside the scope of this

thesis; a review can be found in Jeffrey & Cohen (1971). However, the essence of the phenomenon in relation to infant olfaction will be briefly addressed in the following digression. The aim of this is to highlight the importance of Engen & Lipsitt's findings, in terms of infant olfactory behaviour.

2.13.4. Repetitive stimulation by one odour will eventually result in the subjective experience of loss of intensity (Jeffrey & Cohen, *op.cit.*). This is generally the case in adult subjects; indeed it is probably an everyday occurrence for most people. An example would be of entering a room in which there is a woman wearing a strong perfume. After a variable amount of time, this is perceived as becoming less and less noticeable until the odour becomes imperceptible. However, should the odour be re-applied at any stage by the wearer, the original intensity will be perceived. The generally accepted explanation for this is habituation and a novelty effect. This effect has, however, been termed dishabituation, by Sokolov (1963). One of the reasons for this may be that the perceptual system makes a 'decision' about the significance of repeated stimulations and accords decreasing levels of significance as a function of number of stimulations. The perceived result of this is a decrease in intensity. Such a phenomenon presumably facilitates working in malodorous occupations such as animal glue manufacture, leather tanning and fishery.

2.13.5. Habituation is a useful tool in infant psychology (Pancratz & Cohen, 1970; Rosenblith & Sims-Knight, 1985). The rationale of this is clear. Since pre-linguistic subjects cannot describe stimulus intensity, their dependent measures do so, albeit indirectly. If an infant is habituated to an odour, shown for example by a decrement in an autonomic parameter and then a novel odour is introduced, the response measure will increase. This is the novelty effect. This is interpreted as the perceptual system being able to <u>discriminate</u> between odours. Such an effect is even seen if the novel odour is very similar to the habituating odour, indicating that the response decrement is not due to receptor cell 'fatigue'. Clearly, the main use of the habituation/dishabituation paradigm is to demonstrate <u>discrimination</u> between odours in infants. This has

been applied to infant olfaction research in terms of respiratory plethysmography (Murray & Campbell, 1970; Kendal-Reed *et al*, 1986). Engen & Lipsitt (1965) used stabilimetry as their dependent measure and concluded that odour habituation, rather than receptor cell fatigue, accounted for their findings.

2.13.6. Three years later, this work was set aside in favour of an investigation of intensity perception differences between adults and infants (Engen, Cain & Rovee, 1968). This was a large psychophysical study comparing responses to a series of aliphatic alcohols (see Table 3) in adults and neonates. The authors concluded that infants would respond differentially to the alcohol stimuli according only to the physico-chemical attributes of the stimulus: the carbon chain length. This work was extended by Rovee (1969), who conducted a stabilimetry study of 125 subjects in the first three days of life. This author was able to corroborate the previous study of Engen and co-workers. Rovee's subjects were able to demonstrate fairly fine discriminative abilities between the various alcohols in the aliphatic series (see Table 3). This was interpreted as meaning that infant discriminative ability was much more sophisticated than formerly believed. Such a conclusion was corroborated by a later, cross-adaptation study by Rovee (1972).

2.13.7. These purely psychophysical studies are important milestones in the literature. This is because they can be seen as an attempt to catalogue the basic response repertoire of the human neonate to olfactory and chemosensory stimulation. This work at Brown University did much to define the limits of the human infant chemosensory 'performance envelope', to borrow an aeronautical term. Engen really began the modern era of infant olfactory testing with these carefully controlled psychophysical studies. This work stimulated other research concerned with subtle variations in response.

2.14.1. An example of this would be Murray & Campbell (1970), who were concerned with a discussion of 'state' in infants. The authors used the stabilimetry/respiratory plethysmography technique, with the infants asleep during presentation. The authors

stated that classification of sleep state markedly affected olfactory threshold. This conclusion has important bearing on the practical side of infant olfactory testing. It has been the author's experience with young infants that the time of testing must aim to coincide with the subject's period of maximum alertness. For this reason, the immediate post-prandial period is best avoided when possible.

2.14.2. Another study in the psychophysical tradition is that of Self, Horowitz & Paden (1972). This was concerned with responses of infants in the first three days of life. Dependent measures were "respirometry" and changes in observed behaviour to the odours of asafoetida, lavender and valerian. Self *et al* found that the order of stimulus presentation influenced the results and that subjects also responded to a supposed 'control' stimulus. This latter conclusion well illustrates the dangers of false positives in this kind of research. Similar small-scale psychophysical studies are still continuing and comparable results were found by Shimada, Takahashi, Imura & Baba (1986).

2.14.3. Engen and his team continued their work into the 1970's, at the Injury Control Research Laboratory (Engen & Corbit, 1970; Engen & Moskowitz, 1972). This work used pre-school children (older than four years) in an effort to discover any odour sufficiently aversive across age-groups to ensure that its addition to poisonous household chemicals would prevent accidental ingestion. None was found; suggesting that young children learn odour hedonics as they acquire language.

2.14.4. Engen has continued to contribute to the study of infant and child olfaction into the 1980's. His 1982 book, ***"The Perception of Odours"*** was very important in this area. This work collated and summarised the state of knowledge at the time. All the works of Engen and his collaborators are mentioned in this book, if not discussed at length. He took the opportunity to summarise the work that he and his colleagues had carried out at Brown University, emphasising the psychophysical approach to infant olfactory testing. The main importance of this book to infant olfaction is that it brought

together the diverse strands of knowledge in this area and has done much to stimulate more research in this area.

2.14.5. Engen has continued to publish; mainly review work. A short chapter (Engen, 1986) served to summarise his work on olfaction in infants and children. His recent work (Engen, 1988) concentrated on the hedonic dimensions of odour and how these might be learned. One section discussed infant olfaction, taking the view that neonates show no evidence of hedonic discrimination. He made an important point, crucial to all infant olfaction researchers, that:

> *"Unfamiliar odours will arouse babies because of their novelty and startle value but have no other hedonic properties".*

An orienting, or novelty response is commonly seen in young babies (Lynn, 1967). It ought not to be misinterpreted as due wholly to some hedonic property of the odour stimulus. This makes for cautious interpretation when dealing with odours that adults find pleasant. It would therefore be risky to ascribe the same kind of judgement to infants who respond in an apparently 'hedonic' fashion to the odour. This problem has been addressed by Jacob Steiner, as discussed below.

'Facial Expression' Studies (Group 5, Table 2)

2.15.1. In contrast to the essentially psychophysical studies of Engen and his colleagues, Steiner's work has been essentially concerned with the ethological interpretation of facial expression. To this end, he has been investigating facial responses to odorants and tastants in a wide variety of subjects. His olfaction work has been published in a number of papers (Steiner, 1973; Steiner & Finnegan, 1975; Steiner, 1977) and compiled in a review paper (Steiner, 1979).

2.15.2. This latter paper encapsulated the research philosophy that Steiner espouses. He described it thus:

> *"It can be seen that the human organism evaluates food odours according to their hedonic dimension in a discrimination similar to that by which tastants are selected as acceptable or aversive. This selection, too, is not a result of previous acquisition of experience but rather is a part of a complex innate mechanism. The facial signs emitted by the organism are of communicational value"* (page 263).

Steiner then went on to present a large amount of evidence for this view. His neonatal work involved presenting subjects with odours previously rated by adults as either 'hedonic' (e.g. banana) or 'aversive' (an artificial shrimp flavour). The resulting array of facial expressions were recorded on photographs and videotapes and decoded on a single-blind basis by both experienced and 'naive' raters. The inter-rater reliability coefficient was said to be "perfect" for at least one 'aversive' stimulus. For 'pleasant' stimuli, the highest measure of agreement was for a banana flavour, at r=0.65. It is not made clear whether this correlation coefficient was statistically significant.

2.15.3. Several cases of congenital cerebral malformation were also presented. Subjects, later found at autopsy to possess no identifiable cortical structures superior to the brainstem, were tested with the same method. These anencephalic infants were presented with intra-oral stimuli and demonstrated similar responses to 'normal' infants. One hydroanencephalic subject was also tested with odorants and produced what Steiner terms the typical "nasofacial" response. He cited these subjects as evidence for a special reflex existing in the brainstem. This "gustofacial" or "nasofacial" (for odours) reflex was explained in detail in Steiner (1973) and evidence for its existence in autistic children has recently been offered (Steiner, 1990). The empirical evidence for such a reflex was not really brought into question by Steiner, even though only his own studies and those of Pfaffmann *et al* were cited in this context

(Pfaffmann, Norgren & Grill, 1977; cited in Steiner, 1979). More recent research has, however, extended this paradigm in taste work, using heart-rate as a corroborative dependent measure in infants (Steiner, 1990). This study is reported to have confirmed earlier results. Such a reflex may indeed exist, but it is Steiner's interpretation of the function of such a reflex that is debatable. It should also be noted that odour concentrations in these studies were high. Consequently, these supra-threshold odorants may simply have elicited a 'startle' response because of their power.

2.15.4. His argument, as he described it, is that there is an innate "hedonic monitor" which is 'hardwired' into the human brainstem during gestation. This is difficult to refute because of the nature of the evidence offered. This evidence relied solely upon subjective judgements of facial expression that are reflexive in nature. Observer bias may well have crept in, despite good experimental design.

2.15.5. Unfortunately, the experimental evidence for the 'nasofacial' reflex in human infants rested solely on observer interpretation, which notoriously difficult to operationalise and free from bias. Steiner (1979) proposed that this reflex may be implicated in intercommunication within the mother- child dyad. If this is the case, there is no clear reason why it should be confined to the first few days of birth and then spontaneously diminish. Kendal-Reed *et al* (1986) attempted to replicate Steiner's work using slightly older infants, as discussed in Chapter 4, and Appendix 1. Using very similar methodology and 'hedonic' stimuli, these workers found very low inter-rater reliability using naive raters on a single-blind basis. As far as is known, Steiner's work remains to be independently replicated, as reported by Lipsitt, Engen, Bloom & Jennings (1975; cited in Engen, 1986). This is most unfortunate, because if corroborative evidence for Steiner's theories were discovered, then the whole area of infant preference behaviour would require some re-examination. The concept of an innate, 'hard-wired' hedonic system is fascinating, but histological as well as behavioural evidence would be needed by way of substantiation. Until this is shown, Steiner's work remains tantalising.

2.15.6. Nonetheless, despite some of the methodological and conceptual criticisms of this kind of research, Steiner's work is very important in the field of infant olfaction. His theories provide a necessary and different perspective from those in the purely psychophysical tradition. His approach adds further weight to the view of the infant being olfactorily competent at, or soon after birth. Furthermore, he has amassed more infant data than almost any other researcher this century and his example has doubtless stimulated other workers. Other researchers also take Steiner's view that, for the infant, odour has a semiological or signalling role. It may be that Steiner is really considering stereotypical facial responses as a 'bridge' between pre-cognitive behaviour shortly after birth and cognitive behaviour which develops later. In other words, odour provides an interim communications channel. This concept is discussed more fully below. The notion of communication introduces the concept of 'social olfaction' into the infant arena. This concept in itself requires the use of a well-tried infant behaviour variable; that of preferential behaviour in response to odour.

2.15.7. The idea of preferential behaviour in infants has been used as dependent variable in several studies, notably in the field of infant olfaction by Rieser, Yonas & Wikner (1976). Other, non-olfactory examples[7] are also provided by these authors. This kind of behaviour has always been seen as a reliable indicator in pre-linguistic humans, or animal subjects. Rieser *et al* used this paradigm to investigate olfactory radial localisation. Ammonium hydroxide was used as a stimulus in 20 subjects. Head movements were filmed and then decoded, showing that more subjects turned away from the stimulus than towards it in an effort at avoidance. This assumption that such preferential behaviour can be shown in young infants forms the basis of much work on social olfaction in infants.

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[7] Fantz, Fagan & Miranda (1975), Macfarlane (1975), Prechtl (1958), Turkewitz, Gorden & Birch (1965), Turkewitz, Birch, Moreau, Levy & Cornwall, (1966). The last three studies are cited in Rieser, Yonas & Wikner, (1976).

'Social and Ethological Olfaction' Studies (Group 6, Table 2)

2.15.8. This was used to good effect in important pioneering work by Macfarlane (1975), which considered the role of social olfaction in the young human infant. Macfarlane described two experiments involving neonates given the choice of head-turning toward a clean breast pad or one that had recently been worn by their mother. The results of this study showed an apparent preference for the maternal pad. The second study aimed to show differentiation between maternal and 'alien' mother breast-pads. This resulted in a significant number of older babies showing preferential behaviour for their own mother's pad. This was explained in terms of older infants (up to 10 days old) being motorically able to demonstrate discrimination, whereas younger infants might be able to discriminate but be unable to show it, due to insufficient motor development.

2.15.9. The study of Russell (1976) replicated Macfarlane. Russell briefly reported two experiments, one of which involved infants in a longitudinal design. This study involved differential behaviour with sleeping subjects being presented with cotton swabs. Mothers had previously worn these swabs in their bras, thereby impregnating them with skin odour. Russell described the babies' responses over a six week test period and described the most common response as a "sucking orienting response". However, only older babies gave this preferential response to maternal odour, over that of an alien mother or control swab.

2.15.10. These findings were seen as evidence for a possible maternal 'pheromone' to which the subjects responded differentially and preferentially. However, it would seem that there are a number of flaws in this study. Firstly, all the infants were in a state of hunger during testing, having been deprived of food for the previous three hours. It is unlikely that the subjective sensations experienced by each subject were comparable, as the rate of gastric emptying would be variable between subjects. This would tend to confound between-subjects comparisons. In any case, a hungry infant would not seem to be an ideal choice to demonstrate fine-grain

discrimination abilities. Perhaps the infants' olfactory acuity may have been improved by hunger, but their general discomfort would have made interpretations of behaviour difficult.

2.15.11. Secondly, it has been shown in at least one study (Murray & Campbell, 1970) that physiological state in terms of awake or asleep may govern neonatal olfactory threshold to some extent. If this is the case, a sleeping infant may have a high threshold to relatively low intensity biological odours. Finally, this widely-cited study contained no mention of any statistical corroboration of the findings. It is therefore possible that the 'preferential behaviour' derived from chance. This study has been widely cited in the area of infant olfaction, so it is therefore unfortunate that it is somewhat devalued by methodological problems and offered no statistical support for its conclusions.

2.16.1. The idea that infants will demonstrate preferential behaviour when presented with odours forms the basis of much of the work in another large area of infant olfaction. As mentioned above, social olfaction is an important area for psychologists with an ethological bias. This orientation is concerned with human behaviour in the context of other primates, and intra-species interactions. The viewpoint of social olfaction is that behavioural repertoires seen in other mammals, such as feeding, mating and behaviour with con-specifics, may be generalisable to *Homo sapiens.* These animal behaviour repertoires involve communication by biological odorants that may act as semiochemicals. Doty (1981) summarised this view:

> *"In light of the widespread current interest in the chemical communication of mammals, it is of both academic and practical interest to establish if man, just as most other mammals, can determine information related to gender, reproductive state, emotion, and subspecies or race from body odours. Furthermore, it is of interest to determine if odours are used by infants in recognising their mothers, or by mothers in recognising their infants, as occurs in many other mammals"* (page 352).

2.16.2. Doty presented much evidence in favour of this view in his review paper (Doty, 1981). This remains a comprehensive overview of the current state of knowledge of how odorants are implicated in both disease states and human communication. In terms of infant olfaction, the major studies were reviewed and the paper ended with a call for empirical support for the type of odour-driven 'imprinting' phenomenon seen in other species.

2.16.3. Doty's paper is important because it drew together and synthesised the diverse strands of research in human olfactory communication. He quite rightly pointed out that much more evidence is needed before olfactory intercommunication can be accepted in humans to the same degree that it is in other mammals. Adult humans, with their extremely complex behavioural repertoires, may be much less susceptible to low-level semiochemicals. This is because linguistic and also non-verbal communication provides a far higher capacity channel for information transfer. Whilst not precluding a role for olfactory communication, it might be said that of adult *Homo sapiens* that evolution has provided far better and faster tools for signal-transfer in adults. Perhaps speed and efficiency of intra-species communication is directly proportional to degree of complexity of behaviour? The prices paid by lower animals for olfactory communication are stereotyped behaviour responses to semiochemicals. This is reflected in the large areas of the non-simian mammal cortex being devoted to olfactory processing, perhaps at the expense of more cognitive functions. It would seem that the human brain has moved odour perception to a higher, more cognitive level, in that odour perception is not merely the precursor of a behaviour pattern, but more a stimulator of affect and memory retrieval.

2.16.4. Nevertheless, if it is accepted that humans are an altricial species, then it may be possible that they share, in an olfactory sense, some of the characteristics of other such species. The importance of olfaction to altricial species was summarised by Alberts.[8]

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[8] Cited in Rosenblatt (1983)

> *"Olfactory influences on behaviour are profound and widespread. Neonates, particularly those with limited sensory channels, rely on olfactory input to such an extent that we cannot appreciate their behaviour without understanding the roles of olfaction"* (page 369).

Perhaps the importance of olfaction in the social sense, at least in terms of the mother-infant dyad, may be extended to the human infant. Other workers have advanced this thesis with special relevance to mother-infant interactions involving odour recognition (Porter, Cernoch & McLaughlin, 1983; Kaitz, Good, Rokem & Eidelman, 1987). It is at this point that the studies of social olfaction and those of infant olfaction overlap.

2.16.5. In terms of social olfaction as related to the human mother-infant dyad, research has come from two main teams. These are Porter and his colleagues and Schaal and co-workers. The prodigious work of Porter *et al* is listed below as a footnote[9]. Porter and his colleagues have investigated the social role of olfaction, not only between mother and infant, but also older children and adults. The infant studies are discussed below.

2.16.6. The first of these studies (Porter, Cernoch & McLaughlin, 1983) concerned mothers' ability to recognise their own infants from garments worn during the first six postpartum days. This work discussed the importance of odour in dyadic human interaction, in the tradition of Macfarlane. The authors concluded that:

> *"Chemical cues are continuously present and therefore potentially accessible to the mother. Over the first few days of life, therefore, the odour of an infant may be more invariant than its visual appearance - which can alter considerably during the early postpartum period"* (page 153).

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[9] Porter & Moore, 1981; Porter, Cernoch & Perry, 1983; Porter, Cernoch & McLaughlin, 1983; Cernoch & Porter, 1985; Balogh & Porter, 1986; Porter, Balogh, Cernoch & Franchi, 1986; Porter, Balogh, & Makin, 1988; Makin & Porter, 1989.

The gist of this would seem to be that evolution has selected for a maternal-infant odour recognition ability. This would permit kin recognition during night-time and potentiate any 'bonding'[10] that may occur. Porter *et al* stated that olfaction might be an ideal sensory channel for mothers to use in identifying their infants.

2.16.7. The idea of the 'olfactory communications channel' was elaborated in a review paper (Porter, Cernoch & Perry, 1983). This stated that this olfactory channel is indeed implicated in mother-infant 'bonding'. The authors stated their views thus:

> *"To the extent that characteristic odours facilitate maternal recognition of the neonate, the infant's recognition of the mother, or both, such (olfactory) cues would be involved in the development of mother-infant bonding or attachment".* (page 150).

This does not imply, as the authors stated elsewhere, a critical period when olfactory stimuli are necessary for 'bonding'. Olfactory cues may indeed merely promote neonatal recognition of the mother. It may, in evolutionary terms, be more beneficial if there is no critical period when olfaction promotes 'bonding' with a specific caretaker. If maternal death were to occur in the postpartum period, as commonly happens, a one-to-one irreversible bond, driven by olfactory cues, would be disadvantageous. It is more likely that recognition of the mother is more important, for continuity of care of the infant.

2.16.8. Cernoch & Porter (1985) went on to show that infants are apparently able to discriminate between the axillary odours of their mother and those of 'alien' mothers. This work was elaborated in a more recent paper (Makin & Porter, 1989). Interestingly, only breast-fed infants were able to do this and they were unable to discriminate paternal odour. The authors concluded that breast-feeding infants become rapidly familiarised with their mother's

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[10] This term is given in inverted commas because it is a somewhat nebulous concept in psychology. No satisfactory definition or evidence specifically relevant to the human has yet been found.

"unique olfactory signature". In another paper (Balogh & Porter, 1986), the authors made the very important point that:

> *"It is possible that olfactory cues are of special relevance early in life, before other sensory modalities become pre-eminent and cultural norms militate against the reliance on, and accentuation of, chemical signals"* (page 400).

It is clear that Porter and his colleagues have made an important contribution to infant olfaction. Their insistence on the necessary role of odour in the ecologically valid mother-infant relationship has highlighted the real-life olfactory situation. The work of Porter is somewhat removed from the tightly-controlled psychophysical work of Engen, but still a necessary area of exploration if a complete picture of infant olfaction is to ensue.

2.17.1. Another group working in the same area of social olfaction is that of Schaal and his colleagues[11]. This researcher has provided an ethologically-based overview of the probable biological importance of the olfactory system in human infancy. In an early paper (Schaal *et al*, 1980), he stated that from the fourth day of life, the infant can recognise the*"complexe odorant"* deriving from the maternal breast, areolar and nipple tissue. This work is similar to the preferential behaviour studies described above. Schaal filmed neonates responding to gauze swabs close to the face. Subjects' movements were then later analysed and decoded in great detail. The results of this study were held to demonstrate that neonates can recognise maternal odour from about the fourth day of life. Schaal's studies were methodologically more rigorous than Macfarlane's, but still reliant on the somewhat vague and relatively unsubstantiated method of 'preferential motor behaviour'. Until fairly recently, it has not been possible to address central and cortical activities in infants exposed to odours.

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[11] Schaal, Montagner, Hertling, Bolzoni & Moyse, (1980); Schaal, (1984a&b; cited in Schaal, 1988a); Schaal, (1986); Schaal, (1987); Schaal, (1988a & b).

2.17.2. However, in the field of infant olfaction, Schaal is an important theorist and researcher. One of his major contributions to knowledge has been to write an excellent review paper, which brought together the diverse strands of infant olfactory perception (Schaal, 1988a). Such a review has been needed for a long time and since this is a key contribution to the literature, it will be examined in some detail.

2.17.3. The aim of this paper seems to have been to integrate research approaches and group them according to similar characteristics. One aspect of the research body that was stressed throughout is the social and ethological point of view. The point was made that Man is less 'microsmatic' than was previously thought and that this has implications for human behaviour. Schaal inferred this from animal studies of other altricial mammals. He stated that these mammals all receive information from their environment via the olfactory system - in other words, the olfactory sense has a semiological role for receiving and decoding signals. Indeed, he described the human infant and child as "olfactory receivers".

2.17.4. Furthermore, Schaal considered the idea of the pre-natal development of infant olfaction. The author cited a number of animal behaviour studies, concerning the biasing effect of intra-uterine chemosensory experience on postnatal olfactory preferences. He did not go so far as to suggest that this work is necessarily generalisable to humans, but did suggest ways in which this hypothesis may be tested. This is an intriguing and plausible idea; the view that amniotic fluid can constitute a chemosensory milieu as well as a life-support environment. It is discussed at length in Chapter 3 of this thesis.

2.17.5. The paper then went on to consider evidence for social odour discrimination in older infants and children. The discussion of this section centred on the work of Olson (1981; cited in Schaal, 1988a), which considers infant social cognition. However, Schaal seemed to be linking this theoretical framework to the discriminative faculties, for social odours, of infants and children. Perhaps he was suggesting olfaction as a communication channel for infant social

cognitive information, either as sender or receiver of semiochemically-based signals.

2.17.6. The final section of this paper concerned the ethological 'purpose' of social olfaction, including evidence from pathology and psychopathology. The olfactory perceptual ability of the infant within the mother-child dyad was addressed, particularly the role of areolar odour and 'attachment'. The view was advanced that specific semiochemicals produced during lactation 'regulate' infant physiology, perhaps via a steroid mechanism. This seems to be quite plausible. Anxious mothers, after all, can appear to generate anxiety in their babies; a phenomenon readily observable during 'difficult' breastfeeding sessions. This begs the question as to whether an increase in circulating maternal catecholamines alters her 'odour profile' above the detection threshold of the infant? If so, that may in turn generate infant catecholamine release. A 'fight and flight' semiochemical, perhaps?

2.17.7. In conclusion, Schaal spoke of feature detection abilities for odours in infants. These are part of a "remarkable competence", as he put it, in olfactory terms. He stated that the infant olfactory system is characterised by a high degree of plasticity. He described it, apparently in terms of General System Theory (GST), as an 'open' system. Whilst this is certainly the case, all infant sensory modalities are open systems, to a greater or lesser extent. The application of GST to infant olfaction is an interesting one but it would have to go beyond this level. The application of General System Theory to the field of infant olfaction is discussed elsewhere Chapter 3 of this thesis.

2.18.1. Schaal has written a further paper which expanded on the theme of the importance of the prenatal period in infant olfactory perception (Schaal 1988b). This work extensively discussed the current state of knowledge of the mammalian intra-uterine chemosensory environment. This was then generalised to the human foetus and neonate. The ideas generated by this important paper are addressed in Chapter 3 of this thesis. Recent work in animals (Pedersen, Stewart, Greer & Shepherd, 1983;

Pedersen, Greer & Shepherd, 1986; Smotherman, 1990) and also human foetuses is highly suggestive that the foetal environment is conducive to learning and experience of various types (Hepper, 1990).

2.18.2. It should be emphasised that there are also other workers in this field of social olfaction. For example, Filsinger & Fabes (1985; Fabes & Filsinger, 1986, 1988) discussed the area of social olfaction in relation to the human family. These workers have reviewed the infant olfaction literature, from the viewpoint of mother-infant dyadic interaction. They tended to be somewhat behaviourist when interpreting the studies described above. They stated:

> *"The issue of an infant's recognition of its mother is somewhat less clear-cut. While babies tend to orient toward maternal odour, it is not clear exactly what is taking place, in large part due to difficulties in interpreting the infant's behaviour"* (page 356).

This is an important point, in that, as stated above, the concept of infant recognition of maternal odour rests solely on the observer's interpretation of 'preferential behaviour'. Reliable interpretation may well await a different methodology that addresses cortical events directly.

2.18.3. If such behaviour is mediated primarily by olfactory cues, as Schaal and others believe, then the ability to discriminate with accuracy low- intensity biological odours is a prerequisite. Despite the increasing methodological rigour of studies in this area, such an ability has yet to be reliably demonstrated beyond doubt. The primary reason for this contention is that such studies are wholly reliant on one dependent variable; that of preferential orientation of the head and similar behavioural indices.

2.18.4. There are several alternative hypotheses that might account for such preferential behaviour. For example, differences in infant hemispheric dominance and anatomical asymmetry. These are

described in a paper by Fox (1985), who cited a number of studies which confirm this. In particular, those of Türkewitz *et al* (Türkewitz & Creighton, 1974; Türkewitz *et al*, 1965, 1967)[12] showed anatomically-derived preferential motor behaviour. This may account for the head-turning responses described by social olfaction researchers. Furthermore, infant head movement in the supine position is partly dependent on skull shape, because a prominent occiput may tend to 'anchor' head movements. This might be avoided in future experiments by supporting the subject to provide free movement of the head relative to the body, perhaps with some form of gimbal mechanism. However, such an unnatural arrangement may further confound results. Lastly, other sensory cues at the time of testing may render interpretation difficult.

2.18.5. If this area is to be clarified, it would seem that a less ambiguous dependent measure is needed. One such may be cortical activity in response to odours. Very little has hitherto been known about this area, largely because of the great practical problems inherent in recording brain activity in human infants. These problems had formerly precluded a catalogue of cortical activity in the normal infant, though some work has been done in this area (Lindsley, 1939; Dreyfus-Brisac, Samson, Blanc & Monod, 1958). Furthermore, most interest had concentrated on the EEG pathologies of the neonatal and infant period (Werner, Stockard & Bickford, 1977: Spehlmann, 1981). However, a new technique; Brain Electrical Activity Mapping (BEAM) may help to clarify this area. This technique is described elsewhere in this thesis.

2.18.6. To summarise this section, it is likely that social olfaction is important within the human mother-infant dyad, but the methodology is at present insufficient to demonstrate this reliably. That is not to detract from the excellent work of this school of thought, but its methodological foundations may be, as yet, a little shaky. It may be that a corroborative dependent measure such as BEAM will strengthen the evidence for this fascinating view.

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[12] all cited in Fox (1985)

2.19.1. There has been a clear move over the years away from the view that the human infant is born with either an absent or severely deficient sense of smell. If anything, the pendulum has swung the other way towards considering the infant as olfactorily competent if not macrosmatic. This statement encapsulates the argument that will be developed elsewhere in this thesis - that of '**infant mesosmia**'. The reasoning behind this proposed term is that animals with highly acute, or long-range olfactory abilities are usually described as macrosmatic (Sacks, 1985). Human adults, because of the perceived relative unimportance of olfaction in the sensorium, are often classed as microsmatic. The purpose of the term **mesosmia** is to suggest that infants fall somewhere between the two in terms of the relative importance and capability of their olfactory sense.

2.19.2. In concluding this chapter, it is clear that there have been essentially two main research strands in the area of infant olfaction. The first is mainly phemonenological and concerned with quantifiable changes in physiological measures. It is with this tradition that the present author most closely identifies. The BEAM method described in this thesis is a development of the search for an increasingly precise, quantifiable measurement of the bodily events surrounding olfactory perception.

2.19.3. The other research trend is allied to social psychology and addresses the role of olfaction in social interaction between mother and baby. The trend is not so concerned with precise measurement of physiological parameters. The ethological approach that this trend is based upon is more concerned with such matters as communication and signalling within a dyad that contains one pre-linguistic member. It is true that the two areas are not wholly disparate and both share a common belief that the human infant is an olfactorily competent being. The view that the infant is effectively anosmic, which was common before the nineteenth century, has virtually died out. With greater advances in investigative techniques it is likely that this idea will disappear for good. If the current resurgence of interest in infant olfaction is at all prognostic, then great strides in this area may be expected.

2.19.4. Finally, the suggestion is advanced that a comparative analysis of the techniques in infant olfaction is needed. This has been partly attempted in the work of Schaal, as described above. However, little attempt has been made in the literature to critically discuss just what psychologists are trying to measure. The function of the infant response to odour needs to be placed in its ecological and social setting of everyday meaning for the infant. In other words, a form of hermaneutic approach. The various measurement techniques in infant olfaction need to be critically evaluated in the light of a model concerned with the relevance of odour to the infant. Such a general model has yet to be constructed, despite the first attempt described in Chapter 3 of this thesis. A first step might be a meta-analysis of the literature, as performed in other areas of psychology. However, it is doubtful if there have been enough comparable studies yet to make such an analysis worthwhile. Nonetheless, the study of infant olfaction stands in need of an overview concerned with where we have been and more importantly, where we are going next.

2.19.5. As has been shown in this review, infants appear to demonstrate a degree of olfactory competence from birth and possibly longer. The next chapter in this thesis proposes a model to account for this behaviour and provide more background for the empirical work described in Chapter 4.

CHAPTER 3
A MODEL OF INFANT CORTICAL ACTIVITY IN RESPONSE TO 'BIOLOGICALLY SIGNIFICANT' ODOURS.

"...it appears likely that prenatal experience can be important in the establishment and maintenance of some neurobehavioral structures and functions that are more clearly identifiable in the newborn". Werner and Lipsitt (1981), page 37.

"...the mother reaches and affects the fetus through the early developing fetal sensory receptors for touch, motion, hearing,vision, taste and smell. Relatively little is known about the short- or long-range developmental effects of such stimulation, although this would appear to be a promising area for study". Hofer (1981), page 186.

Introduction

3.1.1. This chapter addresses the theoretical issues underpining some aspects of infant olfactory perception. An hypothesis will be advanced which states that human infants, like many other altricial mammals, are relatively reliant on their olfactory system at birth and for a period thereafter. The generally accepted term in comparative biology for this dependency is **macrosmia**. This term is generally applied only to non-human mammals. The usual definition states that a macrosmatic creature has a very high degree of competence in the 'chemical sense' of odour detection, usually at a distance (Barr & Kiernan, 1983, page 256). Examples of macrosmatic animals are the caniform species; rodents, fish (notably the Atlantic salmon :- *Salmo salar*), most *lepidoptera* species and other invertebrates such as the scorpion (Gaffin & Brownell, 1990).

3.1.2. All these species depend to a greater or lesser extent upon chemical signals for at least part of their behaviour repertoires. These chemicals are either airborne or water-borne and directly influence specific receptors in the olfactory system of a particular

species. Such chemicals are usually considered analogous to the internal chemical messengers of the hormonal system, which are disseminated by the bloodstream. This is because, in invertebrates and some mammals, these chemicals trigger specific behaviours. Those species, like human adults, who are deemed to have a poor or evolutionarily atrophied olfactory sense are usually termed **microsmatic**, from the noun **microsmia** (Barr & Kiernan, 1983).

3.2.1. The hypothesis to be advanced in this chapter states that certain odours may be more meaningful to young human infants than others. The reasoning behind this assumes that human infants can be treated, from a biological point of view, as another altricial mammal. Altricial species are those creatures born in a relatively immature state of physical development and requiring extended nurturing. Evidence from mother-child dyadic interaction, as discussed in Chapter 2, demonstrates the importance of the olfactory sense in the first months of extra-uterine life. Whether or not there is a pheromone, or group of pheromones that infants recognise as being a unique maternal odour signature, it is possible that olfaction plays a major part in the infant sensory repertoire, because of frequent odorous exposure during breast-feeding. This was stated by Peto, in 1973:

> *"The general odour of the mother's body as well as that of the glands of the nipples, of the sweat between and under the feeding breasts, and in addition, of the armpits are the constant concomitants of the baby's feeding"* (page 325).

Some experimental corroboration of this has recently been offered by Sullivan (1990). It is suggested the human infant may in one respect resemble other altricial mammals in the first weeks of life, in that olfaction plays a large role. Such altricial mammals are effectively macrosmatic, at least initially (Rosenblatt, 1983). This is despite the well-documented emerging dominance in the visual modality in the human being (Werner & Lipsitt, 1981).

3.2.2. If this view can be extended to the neonate and young human infant, then it follows that the olfactory system may be maximally oriented to the processing of a narrow range of 'biologically significant' odours. Candidates for odorants that might be 'biologically significant' to the developing organism are maternal odour, breast milk and, by extension, food odour. Such odours would therefore be accorded the greatest degree of cortical processing upon exposure. In the human being, this may be reflected in the amount, location and type of cortical activity. This idea will be discussed in greater detail below in Section 3.6.1. Such an idea might well relate to the special kind of plasticity ('experience-dependent') suggested for the olfactory system by Greenough, and discussed in Chapter 1 (Greenough *et al*, 1987, cited in Bertenthal & Campos, 1987). Synthetic odours such as perfumes and fragrances whose biological significance has not yet been learned will therefore not accorded the same degree of processing by the brain. Hence cortical activity may be of a much lesser degree. However, this has yet to be demonstrated, despite pioneering work using perfumes and fragrances (Van Toller, Behan, Howells, Kendal-Reed, & Richardson, 1990).

3.2.3. The term 'biological significance' is problematical, in that it lends itself to circularity of definition, as many global terms do. Evidence from animal studies demonstrates that certain odours have a high degree of 'significance' in many species (Rosenblatt, 1983). These odours are not merely preferred, but many are assumed to 'drive' distinct behavioural repertoires and have powerful semiological components. In other words, for the young of many species a narrow range of odours has a powerful biological significance. If this is the case for other mammals, why should human infants be so different from other altricial species in this instance? The model being advanced does not suggest that infants remain macrosmatic for the same length of time that other species do. Indeed, the dominance of the visual system soon outstrips that of the olfactory system, which seems to be relatively undifferentiated.

3.2.4. To summarise the model;

1) In the first few weeks and months of life, human infants resemble other altricial mammals in terms of their olfactory sense.

2) Human infants display a degree of olfactory competence during this period, as shown by most of the studies described in Chapter 2.

3) This competence allows the infant to pay maximum attention to 'biologically significant' odours that represent loci of nutrition and protection. These are usually represented by the maternal odour.

4) Maximising attention on the mother's odour, whilst the visual sense is developing sufficiently to allow visual recognition, is a pro-survival attribute.

5) A biological mechanism may exist for pre-natal experience of odour, that accounts for the observed olfactory competence. This would fit in with Carmichael's (1970) law of 'anticipatory function', described below.

6) A 'systems-based approach' might explain this mechanism.

3.3.1. The remainder of this chapter will be devoted to the exposition of this model for early human olfactory competence, as mentioned at the end of Chapter 2. This competence is called '**infant mesosmia**'. The model is based upon General System Theory (GST), which is a philosophical and epistemological system first proposed by the biologist Ludwig von Bertalanffy (1950). The theory was originally designed to deal with complex, biological systems and their inter-relations. A system is generally defined as "an assemblage of parts in relation with one another". Living systems are usually described as 'open', in that they are dynamic and exchange energy with their immediate environment through semi-permeable boundary layers or 'membranes'. At its most basic level, a single cell is a good example of an open system. Energy is constantly generated by the mitochondria following synthesis of novel proteins by the ribosomes. Electrolytes and amino acids are carried across the cell

membrane, thus ensuring a constant flow of energy across the system boundary layer. The cell system will usually lose heat energy to the environment, as well as waste products. Entropy is thus postponed because of this exchange process. In other words, living systems do not have a tendency to 'run down' in the same way that mechanical, non self-sustaining, systems do. Such systems are known as 'closed systems'. They are rare in biology, but spore-forming micro-organisms are an example.

3.3.2. In systems thinking, larger systems are composed of smaller systems, in a hierarchical structure. An example of this could be the human brain. This organ can be viewed as a very complex set of systems and sub-systems, all of which interact. To put it another way, systems within the brain can be viewed as inter-relating and usually inter-dependent in that they are in close proximity and exchange energy. This idea is reiterated by many systems theorists, including Checkland (1981).

3.3.3. This is not to say, however, that a universal definition of a 'system' has been agreed amongst system theorists. In some ways, a 'system' can be defined however one wishes. It should be noted that a system is still an abstract concept rather than a well-defined entity. As Allen and Starr (1982, cited in Checkland, 1988) put it, rather ironically:

> *"What a holon* [synonym for a 'system'] *shall contain is determined by the observer".*

3.3.4. Some 'systems' authors (Checkland, 1981; Morgan, 1986) have a pragmatic approach to GST, in that the highly detailed mathematical infrastructure of the theory expounded by von Bertalanffy (1950, 1968), Klir (1972) and Miller (1978) is given less prominence. Morgan (1986), in particular, speaks of the 'systems approach' in his analysis of organisations. Hence, the idea of 'systems approach', which is more of an epistemological attitude, may be useful. This is discussed below. In other words, the pragmatic orientation implicit in GST is used rather than the formal, propositional aspects of General System Theory. It is this way of

using GST that will be employed in the argument that follows, rather than the formal, mathematical approach espoused by von Bertalanffy and Miller.

3.4.1. To further clarify this view of 'systems approach' to the problem of infant olfactory perception, it is necessary to explain a GST term which is central to the argument. This is '**emergent properties**'. This idea has something in common with one of the Gestalt theories propounded by Wertheimer and his colleagues in the 1930's. Miller (1978) summarised this view:

> *"I have stated that a measure of the sum of a system's units is larger than the sum of that measure of its units.... Because of this, the more complex systems at higher levels manifest characteristics, more than the sum of the characteristics of the units, not observed at higher levels. These characteristics have been called* ***emergents.*** *Significant aspects of living systems at higher levels will be neglected if they are described only in terms and dimensions used for their lower-level sub-systems and components"* (page 28).

3.4.2. When two or more open systems interact, it is not surprising, taking the 'systems approach', that previously unpredictable elements will emerge as a result of this interaction and its necessary exchange of energy. Taking Miller's view a stage further, 'emergents', or 'emergent properties', as they are sometimes called (Ryder, 1990), arise as a **direct result** of inter-systems interactions. Indeed, the characteristics of emergent properties are unlikely to have been predictable from even the most minute examination of the components of each system examined in isolation, as stated by Butler (cited in Miller 1978, page 29). The idea of emergent properties will now be related to the development of the human olfactory system.

3.4.3. The argument will be advanced that the olfactory sense is already reasonably mature at birth and that the human infant is capable of reacting to a range of odours. The reason for this might be provided by Carmichael's (1970) **law of anticipatory function.** This states that:

> *"It is biologically essential to have the structures that make later adaptive responses ready at a period somewhat prior to the time when such reactions must work if the animal is to survive and lead a life that is characteristic of its species".* (Page 449. Cited in Werner & Lipsitt, 1981, page 61).

This would provide one explanation of the apparent olfactory competence shown in the studies described elsewhere in this thesis, in Chapters 2 and 4. Studies of intra-uterine behaviour have shown that much post-natal infant behaviour can be demonstrated pre-natally (Werner & Lipsitt, 1981; Bremner, 1988, pages 25-31; Hepper, 1990). The reasoning behind the view that the 'systems approach', combined with Carmichael's law, can be used to explain infant olfactory competence is as follows.

3.4.4. It has been shown from human embryological studies that the developing foetus exhibits differentiation in the olfactory system at an early stage (refer to Table 1, Chapter 1). This indicates the possibility of early function in the olfactory receptors. If the olfactory receptors and their associated neural 'wiring' are functional at this time, it is possible to consider them as a system. They are therefore set up to receive information from their immediate environment. This environment is the amniotic sac, which is part of the larger maternal system. Therefore, in broad GST terms, two or more open systems are interacting. The point to clarify at this stage is that the infant olfactory system is not <u>conceptually</u> different from any other sensory system. It is an open system, in which the human organism abounds. Energy is exchanged at the boundary layer, which, in terms of the olfactory system, means that molecular energy from the immediate environment constantly flows into the system.

3.4.5. Why should the human olfactory system display such apparent maturity at birth, over the other sensory modalities? It is certainly the case in other altricial mammals that they display a striking peri-natal competence in the olfactory system (Rosenblatt, 1983). The evidence offered in this thesis suggests that the human animal may have a similar, though perhaps less acute ability. One explanation for this may be that of pre-natal experience of chemical signals which influence the chemosensory system. This was even suggested as early as 1429 by a poet called Heinrich von Louffenburg (cited in Kessen, 1965):

> *"Therefore the child should delight in taking its mother's breast. On that it subsists better and without harm than on that of any other woman, because it became accustomed to it in the mother's womb"* (page 2).

3.4.6. It is possible that chemical traces from odorous materials ingested by the mother may cross the placental 'barrier', enter the amniotic fluid and then stimulate the developing olfactory receptor cells in the human foetus. This is claimed by some workers, who postulated this mechanism in rodents (Coppola & O'Connell, 1989; Reasner & O'Connell, 1990).

3.4.7. The fact that these odorous molecules are dissolved in the essentially aqueous environment of the amnion is no handicap to stimulating the olfactory receptors. After all, in the postnatal environment, odorous molecules have to be dissolved in the olfactory mucosa before activating the olfactory receptor cells, as described in Chapter 1 (Le Magnen, 1969; cited in Schaal, 1988a). The prenatal and postnatal situations are not dissimilar at the receptor level and it is hence possible that the amniotic sac constitutes a chemosensory environment. This environment may allow pre-natal exposure to odorant molecules; hence stimulation of the developing human olfactory system. This, of course, has yet to be demonstrated. One way of doing so is suggested in Chapter 5.

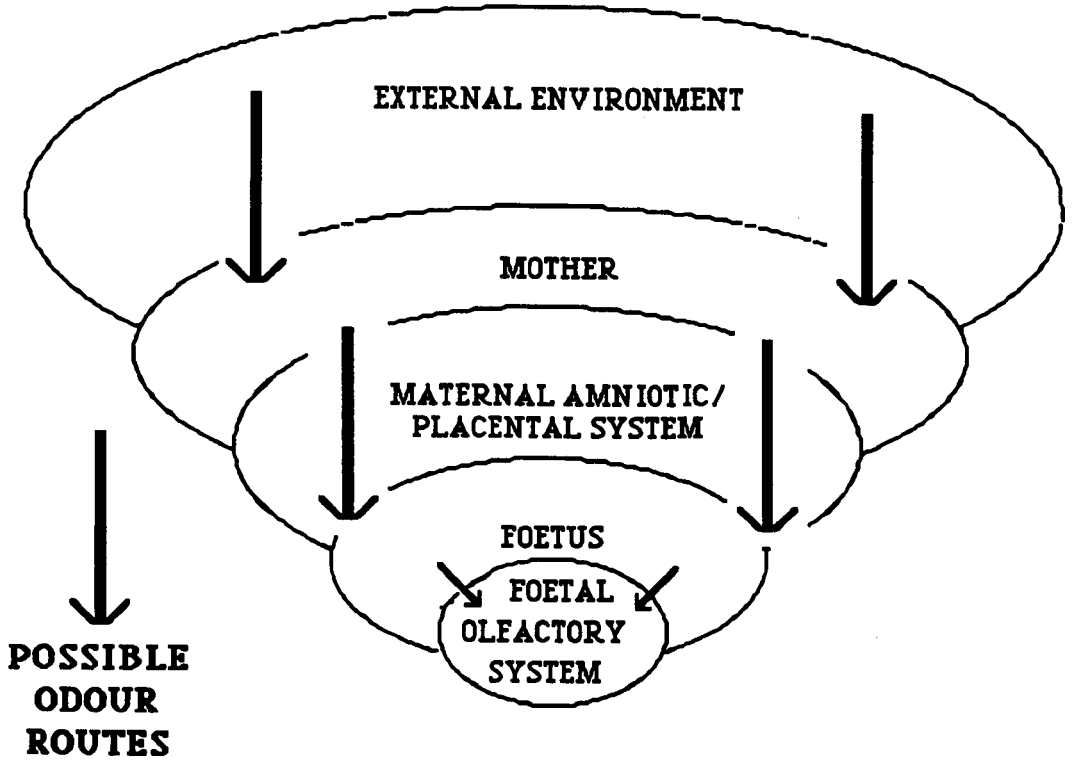
3.4.8. It should be noted that, as far as is known, there is no evidence for what the odorous constituents of this environment are.

Furthermore, the possibility must also be borne in mind that the placenta may act to attenuate the concentration of any odorous molecules reaching the foetal olfactory system. This is, at present, an unanswered question, though some work has been done on placental permeability to certain chemicals (Willis, O'Grady, Faber, & Thornburg, 1986; Thornburg, Burry, Adams, Kirk, & Faber, 1988). No research is available in the human to lend support for actual placental attenuation of non-teratogenic odorous molecules.

3.4.9. A further question to be asked is: what contribution does the foetus itself make to its own intra-uterine chemosensory environment? When the foetal kidneys begin to produce urine, it is possible that metabolic breakdown products of all kinds are released into the amnion. This brings into mind the intriguing question of whether foetuses with genetic errors of metabolism (phenylketonuria, for example) affect their own olfactory systems in a reciprocal fashion by a process that might be called 'auto-odorisation'. It is known that certain diseases of neonates produce characteristic body odours (Mace, Goodman, Centerwall, & Chinnock, 1976). However, because it is not yet known what odorous constituents are present in the amniotic fluid, it is speculative to attempt precise attribution of sources to either foetus or mother.

3.5.1. If the whole situation of biological relationships between mother and foetus is looked at from the 'systems approach' then the relationships between the various systems involved becomes clearer (see Figure 1, below).

FIGURE 1: A DIAGRAM TO ILLUSTRATE POSSIBLE INTER-SYSTEMS RELATIONSHIPS AND ODORANT ROUTES



In the prenatal situation, the 'maternal system' is influenced by the external odour environment in which she lives. An analysis of all the energy exchanges and inter-relationships involved here would be over-complicated. However, it is clear that the mother inhales and absorbs odorous molecules via her own olfactory system.

3.5.2. The fate of these molecules and their metabolites is unclear, despite recent work on enzyme degradation systems in the olfactory epithelium (Jenner & Dodd, 1988). However, it is not unreasonable to suggest that, with constant maternal exposure to numerous environmental odorants, some quantity reaches the maternal bloodstream and possibly the foetal circulation[13]. Food-

---

[13] It is known that chemical pollutant exposure during pregnancy can have effects on the subsequent behavioural development of the infant (Hofer, 1981, page 187). These, or their breakdown products following maternal hepatic detoxification, presumably enter the foetal system either haematogenically, or via the amnion.

based odorants, derived from maternal diet also reach the mother's digestive system. An example might be allyl sulphide, the odorous constituent of garlic. The end result of this ingestion via the maternal digestive system is that nutrients are transferred to the circulatory system (Hofer, 1981, page 187). These serve to nourish not only the maternal system, but by means of the specialised placental/amniotic system, they also nourish and support the developing foetus. Hence any odorous molecules that cross the placental 'barrier' (little is known about this area) have at least the chance of directly stimulating the developing foetal olfactory system. This is known as the 'haematogenic' route (Hofer, 1981; Schaal, 1988a; Hepper, 1989) for odorous molecules. This route has already been hypothesised in an elegant paper by Schaal (1988b).

3.5.3. The following section proposes an hypothesis. If one assumes that odorous molecules of a specific molecular weight and charge are ingested by the maternal digestive system, then a proportion should reach the maternal bloodstream. These molecules survive the digestive process intact because of their molecular weight, or some other physical characteristic and pass through the circulatory system to the maternal side of the placenta. At this point, again due to some physical characteristic, similar to that exhibited by alcohol breakdown products or teratogenic drugs, they enter the foetal circulation as well as the amniotic fluid.

3.5.4. These molecules are then able to directly stimulate the foetal olfactory receptor system. In this example, the foetal system is more than about 28 weeks old, the nostrils are patent and many of the olfactory receptors are functional. This is corroborated by the work described in Chapter 1. At this point, receptor potentials are generated and transmitted via action potentials to the developing neural circuits in the rest of the brain associated with olfactory perception. If specific molecules are a regular constituent of the maternal diet and present in large numbers, then two open systems will exchange energy on a regular basis.

3.5.5. If this situation occurs on a regular basis over a protracted period of time, perhaps during the final trimester of pregnancy, then two or more open systems will have interacting for this period. The conditions are thus right for the development of emergent properties. The diagram above (Figure 1) provides a representation of how the human situation might be represented as an hierarchical arrangement of systems. The notion of hierarchies in systems relationships has been suggested by system theorists (Checkland, 1981). A hierarchy is important in considering the relationship between maternal olfactory and alimentary systems and the foetal olfactory system. This is because odorants probably have to follow hierarchical pathways, rather than influencing the foetal olfactory system directly.

3.5.6. Because of the prolonged inter-system interactions, the systems may interact synergistically (Checkland, 1989, personal communication). The net result of this synergy would be that the foetal olfactory system may undergo accelerated maturation. This maturation is therefore the 'emergent property' of the inter-systems interaction. The term 'maturation' is used in its biological sense of neuronal interconnections occurring. This maturation occurs at faster rate, relative to the other sensory systems that have not had the same prolonged stimulation benefits. As described in Chapter 1, the mammalian olfactory system is noted for its high degree of 'plasticity'. There is no evidence to suggest that the developing human olfactory system is any less plastic. Such an adaptation might well have pro-survival characteristics.

3.5.7. Any enhanced olfactory competence might thus increase species survival because of its effect in augmenting the infant-mother bond. The reason for this might be concerned with the type of odours that are best perceived by the precociously mature infant olfactory system. If satisfactory 'bonding' within the mother-infant dyad is pro-survival in the evolutionary sense, then the infant system might respond maximally to the 'biological', low-intensity odours produced by the mother during lactation. This might be analogous to the nipple-recognition demonstrated by rat pups

(Teicher & Blass, 1976). There is indeed some evidence to suggest that this is the case, as described in Chapter 2.

3.5.8. It is not suggested, of course, that human infants are truly macrosmatic in the same sense as rat pups. However, it seems possible that they are somewhat less than macrosmatic and somewhat more than microsmatic. Hence, the term **mesosmatic** seems to be appropriate. The sense behind the coined term **mesosmia** is to convey the idea of infants falling at the half-way point along the spectrum where macrosmatic species are at one end and microsmatic species at the other. This idea would be hard to test empirically, as it would necessarily involve cross-species comparisons. At present, the term '**infant mesosmia**' must therefore remain a concept to help explain some observed phenomena.

3.5.9. There is some evidence, reported elsewhere in this thesis (see Chapters 4 and 5) that this mesosmia lasts at least until the first twelve weeks of life. From anecdotal evidence, as well of studies of infant visual perception, it appears likely that the visual system begins to outstrip the olfactory system at about this time in terms of sophistication and discrimination (Werner and Lipsitt, 1981). Perhaps 'infant mesosmia' is a transient ontogenetic stage designed to enhance survival while visual dominance is developing and becoming established. Nonetheless, the olfactory system may still be important during infant and child development as a low-capacity information channel implicated perhaps in social relationships. This has been suggested by a number of workers (Doty, 1981; Filsinger & Fabes, 1985). It is also possible that olfaction acts this way as a pre-cognitive system. In other words, before the infant has developed sufficient cognitive systems to recognise and interact with the mother, olfaction serves as a precursor system. This has been suggested by Balogh & Porter (1986).

3.6.1. Another way of testing this concept of 'infant mesosmia' would be histological examination of foetal olfactory tissues to asses the degree of neuronal interconnections present at, or just before

birth. This would present formidable practical difficulties in obtaining suitable human material for examination, because of the ethical considerations involved. This especially applies to aborted or stillborn foetuses. However, such work could be done on other mammals, although the degree of applicability to the human situation is debatable. Nonetheless, work is in progress on assessing the amount of odorous material that is orally ingested and subsequently detected in the amniotic fluid of the sheep (Schaal, personal communication, 1989). In humans, there is a similar but non-permanent technique called amniocentesis. This involves surgically removing a small sample of amniotic fluid as a diagnostic procedure to assess, amongst other things, genetic abnormalities in the developing foetus. Such a technique could also be used to test the model of 'infant mesosmia' and pre-natal odour experience.

3.6.2. There is already some very preliminary evidence to reinforce the notion of a haematogenic route for maternally ingested odorants in humans. A small-scale study has suggested a differential response in breast-fed infants to breast milk containing garlic products. This followed garlic capsule ingestion by the mother (Mennella & Beauchamp, 1990b). If garlic metabolites reach the breast milk, then they may also reach the foetal side of the placenta by the haematogenic route. This possibility, in combination with amniocentesis, could be used to test the idea of pre-natal odour experience in the human infant. It is even possible to speculate about how this might be done.

3.6.3. It is proposed that a similar study to that of Mennella & Beauchamp could be carried out, in which certain mothers were asked to include an odorous food product in their diet during the final trimester of pregnancy. These mothers would already be candidates for routine amniocentesis, perhaps because of their obstetric classification as 'elderly primagravidae'. A small quantity of the amniotic fluid could be used for an assay to detect the breakdown products of the dietary inclusion. The infants could then be tested for some kind of differential, even preferential response to this chemical in the first few days of life. If this were shown, then it would lend support to pre-natal odour learning. However,

histological evidence of precociously mature olfactory receptor cells would also be needed to support the 'emergent properties' part of the model.

3.6.4. However, such a dietary inclusion study would still rely on the dependent measure of preferential behaviour response. As has been discussed in Chapter 2, this is often hard to interpret. More refined techniques are now becoming available. One of these is the Brain Electrical Activity Mapping (BEAM) method described in this thesis. If, as the experimental evidence offered in this thesis suggests, this technique can identify cortical response to odour, then a more reliable technique may become available.

3.6.5. Such work may show a qualitative difference between the type of 'biologically significant' odours described above and synthetic odours. If this were shown, it would lend support to the hypothesis that 'biologically significant' odours are perhaps cortically processed in a different way to those whose significance has not yet been learned either pre- or post-natally. As mentioned above in Section 3.2.2., the odour of breast milk might be classified as significant to a neonate. Elements of its composition might have been learned *in utero*, through the haematogenic route, as described above.

3.6.6. By extension, it might be argued that food in general might have an innate significance to infants. It is even possible that post-natal food preferences, when they develop in the first years of life, might have been influenced by pre-natal chemosensory experience. This latter hypothesis would be difficult to test without a large-scale longitudinal experiment. However, using BEAM technology, it was seen as possible to test some ideas about food odour, its biological significance and cortical processing in infancy. In the light of the model proposed above, it was hypothesised that human infants might show differential cortical activity to food odours, because of their biological significance. The empirical work to test this view is described in the following chapter.

CHAPTER 4
A DESCRIPTION OF EXPERIMENTS CARRIED OUT FOR THIS THESIS.

Introduction

4.1.1. Because of the ambiguities inherent in some of the infant testing techniques described in Chapter 2, it was decided to assess a relatively new psychophysical method for addressing infant olfaction. This chapter will address all the experimental studies carried out for this purpose. It is divided into a **Pilot Study** and then the main **Experimental Studies (1), (2) and (3).** The chapter describes experiments using the Neuroscience Brain Imager, which employs the Brain Electrical Activity Mapping (BEAM) technique. The BEAM method had previously been used for adult olfactory testing (Van Toller, 1987; Van Toller, Kendal-Reed, & Sleight, 1987, 1989; Van Toller & Kendal-Reed, 1990, Van Toller *et al*, 1990). This derived from earlier research, using adult subjects, that showed odour to be capable of modifying human EEG (e.g. Finzenkeller, 1966; Allison & Goff, 1967; Kobal & Hummel, 1988, 1989; Lorig & Schwartz, 1988; Lorig, 1989; Yoshida, Saito, Lida, Yamamura & Kanamura, 1989). No previous research was available, as far as is known, to show the effects of odour on human infant EEG.

4.1.2. It should therefore be emphasised that the BEAM technique, as far as can be ascertained, had never before been used to test infant olfactory response. The studies described below are therefore sub-divided into two parts. The **Preparatory Phase** is described in Appendix 3, where the development of all the detailed operations needed to test infant subjects with BEAM is described. The **Experimental Phase** described below contains the main descriptions of the experimental work, referring as necessary to Appendix 2 (Technical Appendix) and Appendix 3 for fine details.

4.1.3. A parallel study using an older measurement technique, Respiratory Plethysmography (RP), is described in **Experimental Study (3).** Also included is a brief summary of separate and preliminary infant studies carried out in the USA using the RP

technique, and that of facial expression measurement (see Appendix 1). This work was a partial replication of the studies of Steiner (1979). The main findings of these preliminary studies are summarised below (Kendal-Reed *et al*, 1986). Subjects were tested repetitively with two odours (anise and almond) and facial expressions were recorded. These expression were later decoded by naive raters in a single-blind design. Poor support was found for the hypothesis that infant facial expressions, at the age of three months, could be used as dependent measures for response to these odours. Respiratory plethysmography was used in a subsequent study and elicited a significant 'novelty' response to the same odorants. This is in contrast to later work at the University of Warwick using a different paradigm, but the same basic technique. This showed no difference between a control condition and an odour condition.

EXPERIMENTAL PHASE

Preamble

4.2.1. As the work summarised above has shown, as well as the studies discussed in Chapter 2, there are limitations to some of the measurement techniques employed in the study of infant olfactory response. The subjective element in facial expression work and the somewhat doubtful results of respiratory plethysmography conspired to produce a need for a more central, less ambiguous measurement technique. Early work with adult subjects at the University of Warwick (Van Toller, 1987) had suggested that this BEAM method would also be useful for measuring central, cortical responses in infants exposed to odours. In this way, purely behavioural responses, with their inherent ambiguities of interpretation, could be by-passed in favour of recording real-time EEG from the surface of the brain, using a special infant-size electrode headcap (see Plate 1).

4.2.2. It should be made clear at the outset exactly what data were being addressed in the work for this thesis. The numerical values produced by the Imager were **amplitude data in microvolts**. Every electrode in each 'frame' of recorded data produced an amplitude value. This was the result of summated

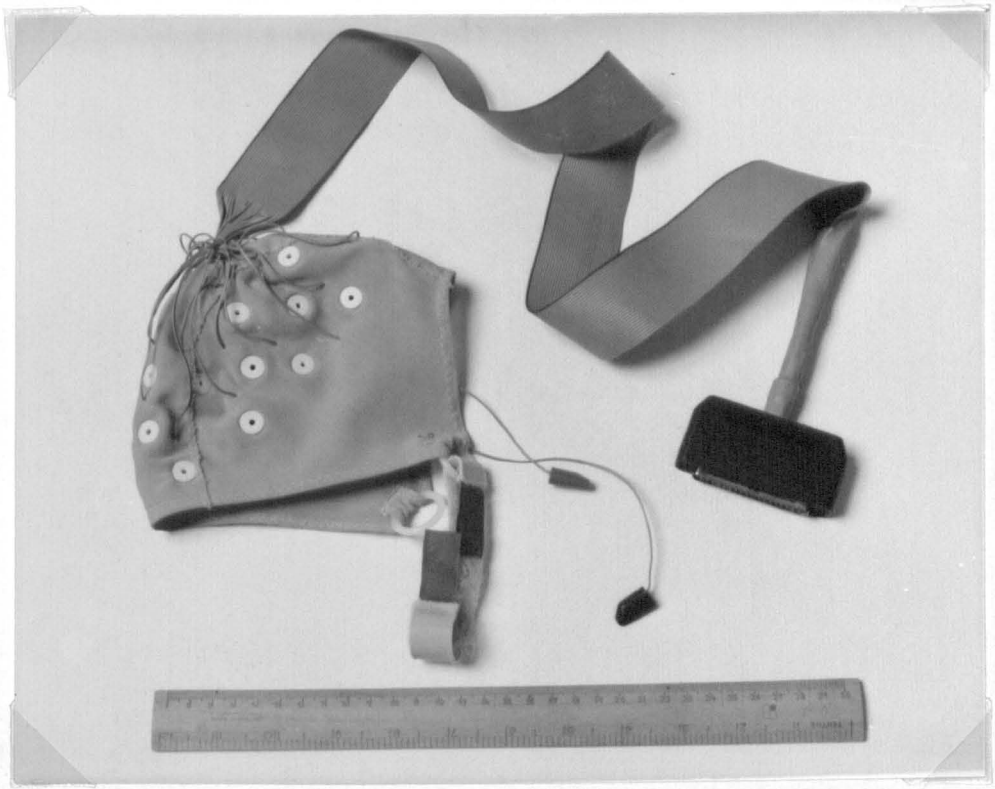


PLATE 1:

THE INFANT ELECTRODE HEADCAP. THE TWO WIRES IN THE CENTRE OF THE PICTURE ARE FOR THE REFERENCE (EARLOBE) ELECTRODES.

cortical output over the 2.56 seconds that each frame lasted. Furthermore, because of the great within-subject data variability, each subject acted as their own control. Technical details and further discussion of these topics are to be found in the Technical Appendix (Appendix 2).

4.2.3. It was anticipated that this technique, if indeed it were usable in small infants, might shed more light on how infants process odours. Hence, a pilot study was set up to test the feasibility of large-scale infant testing. This was done when the necessary preparatory work, described in Appendix 3, had been set in motion or completed. It should also be emphasise that experience with adult subjects in this technique had revealed that the method of data analysis in BEAM needed to be improved upon. The qualitative dimension of the EEG data, in the shape of the topographic maps, had already been explored. However, as discussed in Appendix 2 (Section A2.5.2.), the maps did not tell the whole story. In particular, they did not really offer any way to test null hypotheses. Therefore, part of this chapter will describe attempts to find a suitable inferential parametric analysis technique that could be used in this way.

4.2.4. Because it was hoped that a usable data analysis technique would be found, two main experimental hypotheses were formulated. These hypotheses were that: a) BEAM could be used to show significant differences between a baseline condition and an odour condition across subjects, b) cortical response patterns would differ significantly according to the odour type used.

Pilot Study

Subjects

4.3.1. The population for this work was all mothers who had registered a live birth in the catchment area of Coventry Health Authority. The sample comprised up to 10% of this. These parents were recruited by means of a direct-mail shot described in Appendix 3, Section 1. Twenty-six infant subjects (16 females and 10 males) were recruited for the Pilot Study. No subsequent analytical categorisation on the basis of gender was made in this or other

studies. This was because there was no convincing infant evidence for the type of gender-related odour sensitivity differences reported in older children by such workers as Koelega & Köster (1974). The subjects' ages ranged from 10 to 20 weeks from birth, with a mean of 13.8 weeks. As discussed in Appendix 3, this age group was chosen for a number of reasons. Subjects had generally trouble-free obstetric and perinatal histories. Apgar scores, a standard obstetric measure of post-natal viability use in the UK were not however asked for (Apgar, 1953). Babies born by Caesarean section were included in this testing group. Mothers gave details of dietary intake and a record was made whether breast- or bottle-feeding was used. However, subjects were not subsequently grouped according to this criterion. The mothers were asked if their child had an upper respiratory tract infection. It was not found necessary to exclude any subjects on this basis. All subjects had been fed in the previous four hours; a few in the hour prior to testing. This was to ensure maximum subject compliance with the testing procedure.

Materials

4.3.2. All subjects were tested using a Neuroscience Series III Brain Imager. Details of this device, which can be seen in Plate 2 below, and its use are given in the Technical Appendix (Appendix 2), and Appendix 3, Section 2. The computer settings for the Brain Imager were also as described in the Technical Appendix. As additionally described in this Appendix, efforts were made to reduce the high skin impedances found in infant subjects. This was not generally successful. It should therefore be made clear that all BEAM data reported in this thesis were recorded with impedances greater than 4 kΩ . However, no recording was ever started until the EEG waveforms on the monitor screen had been checked for the lowest possible amount of artifact. Indeed, it was only when the waveforms were judged to be as artifact-free as possible that recording began. The importance of this is further discussed in Appendix 2, Section A2.1.2.

PLATE 2:
NEUROSCIENCE SERIES III BRAIN IMAGER. ABOVE AND TO THE RIGHT OF THE KEYBOARD CAN BE SEEN THE OPTICAL DISK DRIVE AND PtS DATA DOWNLOAD SYSTEM.

Stimuli

4.3.3. Stimuli were as listed below. All were baby foods provided by Cow & Gate Limited, with the exception of Stimulus 9, which had been produced for earlier studies.

1. **Fish & Tomato Sauce.**
2. **Chocolate Pudding Dessert.**
3. **Fruit Delight Dessert.**
4. **Apple & Orange Dessert.**
5. **Banana Dessert.**
6. **Beef Dinner.**
7. **Spaghetti Bolognaise Dinner.**
8. **Strawberry Fool Dessert.**
9. **5-alpha-androstenone (an odorous steroid).**
10. **Empty cup (control).**

N.B. During testing, the order of presentation of the odours was shuffled between subjects, to prevent order effects from obscuring interpretation of results.

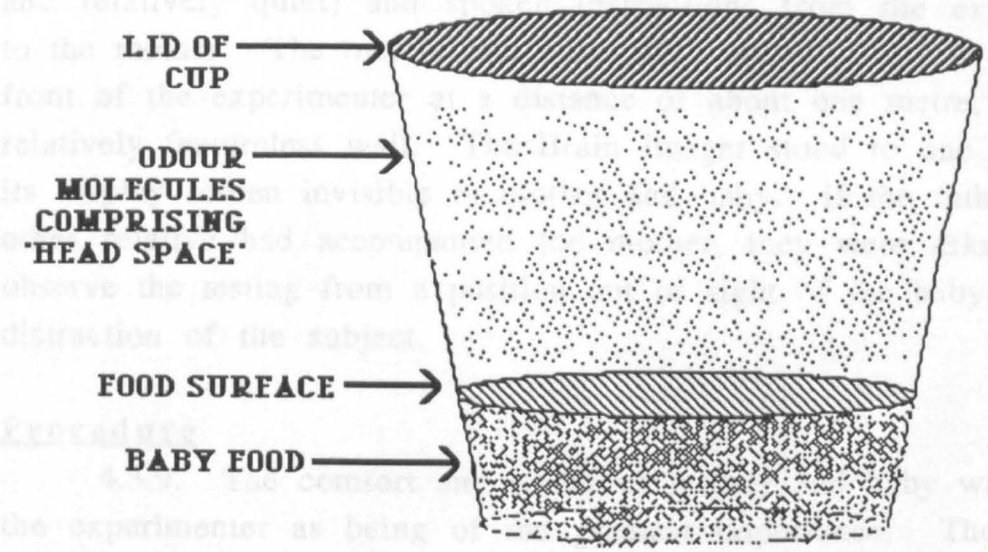
4.3.4. These particular types were chosen because they were approximately iso-intensive odours, meaning that they were perceived as being of similar strengths by adult raters. The need for iso-intensity stems from comparing the responses between various odorants (Van Toller *et al*, 1985). If they are not as nearly iso-intensive as possible, then cross-comparisons of subjects' responses are less valid, because stimuli of a similar intensity are not being compared. Iso-intensity in complex odorants is, of course, a somewhat subjective dimension, even for adults. Furthermore, there is no experimental evidence as to what might constitute iso-intensity for babies in the test age-group, because of a lack of threshold data for food odours.

4.3.5. The baby food stimuli were taken from a fresh or previously opened, refrigerated jar. For reasons of microbiological safety, no food that had been kept under refrigeration for longer than 48 hours was used. The jars of baby food were removed from

the refrigerator and left at room temperature for one hour prior to use. This was to ensure that volatiles would be at the right temperature to comprise the 'head-space' described below. For presenting the stimuli, it was decided to use disposable plastic stimulus containers. The reason for this was to avoid the time-consuming washing and drying of glass containers encountered in earlier studies (Van Toller *et al*, 1985). Stimulus containers were used once and then discarded, thus avoiding the problem of odour residue in the containers.

4.3.6. Stimuli comprised one spoonful (approx. 20 grammes) of baby food, decanted into cups made of very low-odour plastic (50% general-purpose polystyrene, 50% high-impact polystyrene). Each of these cups had an internal volume of 250 ml. Following preparation, all stimulus containers were capped with tight-fitting plastic caps and allowed to stand at room temperature for at least one hour until used. The reason for this was to allow a large 'head space' of odour to develop, whereby any volatile elements present in the food would rise above the surface. This ensured that, on uncapping beneath the babies' nose, a relatively large volume of food odour would be available for inspiration by the subject. This situation is represented in Figure 2 below.

FIGURE 2: DIAGRAM TO ILLUSTRATE 'HEAD-SPACE'



4.3.7. The odorous steroid (5-alpha-androstenone:- see Table 3, Chapter 2) was chosen as a test stimulus because it is a likely component of human odour (Amoore, Pelosi, & Forrester, 1977; Gower, 1989). Infant recognition of maternal odour has been experimentally demonstrated, as described in Chapter 2. The 5-alpha-androstenone stimulus was prepared as 50 microlitres of solution (1mg/ml in ethanol) pipetted onto a perfumer's smell strip and allowed to dry completely in a fume-cupboard. This smell strip was then placed in a stimulus cup and capped to allow 'head-space' to develop, as previously described. A dry smell strip was used in the same way to provide a 'blank', control stimulus. The testing equipment and environment were as described below.

Experimental environment

4.3.8. All testing was carried out in a suite of rooms in a large, airy laboratory adapted to provide a low-noise electrical environment. This was necessary because of the great sensitivity of the brain imaging equipment used. Any unshielded electrical apparatus caused interference, which was recorded by the Brain Imager. The laboratory was in a quiet location and maintained at

approximately 20 degrees Celsius. The laboratory was lit with normal fluorescent lighting, but this was also shielded with metal grids to prevent electrical interference. Ambient noise was limited to muted environmental sounds, equipment cooling fans (all internal and relatively quiet) and spoken instructions from the experimenter to the mother. The mother and baby were seated in a low chair, in front of the experimenter at a distance of about one metre, facing a relatively featureless wall. The Brain Imager stood to one side, with its display screen invisible to mother and baby. If the father, or other relative had accompanied the mother, they were asked to observe the testing from a position out of sight of the baby, to avoid distraction of the subject.

Procedure

4.3.9. The comfort and safety of mother and baby was seen by the experimenter as being of the greatest importance. Therefore, once the parents had arrived in the laboratory, a detailed briefing was given, including a description of the procedural aspects of the study method. The mothers would be shown the Brain Imager in operation and allowed to smell the odour stimuli. The following aspects were emphasised to all parents. Firstly, all parents were given the explicit option, as part of the briefing, of terminating the study at any time they wished. Later, the safety systems contained within the Brain Imager were explained, using non-technical terms. Finally, it was made clear that the child would not leave the mother at any time, nor would the child actually ingest the stimuli and that all information recorded in connection with the study would be kept strictly confidential.

4.3.10. The experimenter explained that the child would be measured for a headcap (see Plate 1, above) and the mother was given the opportunity to examine this. In fact, at each stage of testing, it was explained what was about to happen and why. Following any questions, parents then gave written, informed consent to participation in the study on behalf of their child. If the mother needed to feed the infant or change the diaper, then an opportunity was provided for this in a separate, private room. The infant would then be seated comfortably on the mother's lap,

supported in whatever position was best tolerated. This would often be the same as if for breastfeeding. The testing situation can be seen in Plate 3, below.

4.3.11. It should be made clear at this point that an experimental assumption was made in order to allow the mother to hold her child throughout the experiment. This assumption was that the babies had become habituated to any maternal odours, because of this close proximity. Such an assumption, though not supported empirically, was felt to be justified. Previous experience in infant work had shown that mothers were more reluctant to allow testing if they were separated from their baby. The effect of such separation on infant compliance might have jeopardised data collection by its effect on cortical activity, though no information was available on this. On balance, it was therefore decided to test babies whilst they were held by the mother. Furthermore, the experimenter wore a plastic glove during testing to conceal any odours originating from his skin. These gloves had been allowed to stand at room temperature overnight to allow dispersal of odours derived from packaging or manufacture. Some faint residual odour was noticeable, but this was not considered to be a confound as all subjects were tested whilst the experimenter wore the same type of glove, so this same condition obtained between all subjects.

4.3.12. The infant's head was then measured for a headcap. Further details of this technique are to be found in the Technical Appendix (Appendix 2). A suitable size was fitted loosely to assess whether the subject would tolerate it. If one would appeared satisfactory, then a standard fitting technique would be used. This technique is described in greater detail in Appendix 3, Section 2. If no headcap could be found to fit satisfactorily, or the subject was intolerant of the headcap, then the parent was asked if the child could participate in the parallel Respiratory Plethysmography study described later. The scalp would be gently wiped with a small quantity of ethyl alcohol on cotton wool and allowed to dry. This aimed to lower skin impedance, as discussed earlier. Then the earlobe electrodes would be fitted and gel inserted. Finally, the headcap would be secured on the head, by means of a chin-strap,

and electrode filling would commence. Because of the paramount need for safety, no abrasion of the scalp was employed to reduce the electrode impedances. As might be expected with infants, a considerable number of subjects decided, at this or an earlier point, to cease cooperating. If they could not be soothed in a very short time, the procedure was abandoned by the experimenter. On the occasions that this occurred, every effort was made to reassure the mother that this in no way implied any failure. In most cases, it was quite clear that the infant disliked the feel of the headcap or gel insertion. No attempt was made to re-commence headcap fitting, though mothers were asked if they wished to allow participation in the RP study.

4.3.13. Once the headcap had been securely fitted, it was connected to the Brain Imager in order to check that the system was operating correctly. This operation allowed the experimenter to check that satisfactory waveforms could be recorded. In many cases, some electrodes had to have electrode gel re-applied to ensure a reasonable signal.

4.3.14. When all systems were functioning satisfactorily, testing was commenced. This was carried out according to a standard regime. During recording, mothers were asked not to move and to avoid social interaction with their child. Recording would commence when the subject appeared settled. It should be noted that there was considerable variation of the definition of 'settled'. In some cases it meant 'less mobile than a minute ago'. In others it meant that the subject had been played with, talked to, generally soothed or even fed again over a period of fifteen minutes. Some subjects were almost somnolent, with eyes closed and regular breathing. No attempt was made to define 'state' because of this wide variation. However, most subjects were at least relatively immobile when recording commenced.

4.3.15. When the experimenter judged the subject was ready, the Brain Imager was switched into 'record' mode. No increase in noise level from the Imager was apparent that might have cued the subject. Five frames (2.56 seconds to each frame) were recorded

PLATE 3:

MOTHER AND BABY IN THE TESTING SITUATION FOR EXPERIMENTAL STUDY 1. NOTE THE EXPERIMENTER'S GLOVED HAND, LEFT OF PICTURE, HOLDING THE ODOUR STIMULUS CONTAINER. THE DEVICE TO THE BABY'S RIGHT IS THE PRE-AMPLIFIER FOR THE BRAIN IMAGER.

with no odour stimulus to provide baseline activity recordings for control purposes. It should be noted that the experimenter was cued to present or remove stimuli by a small display on the Imager's monitor. This counted frames as well as time, so stimuli were presented or removed at the moment when the frame counter changed. It required considerable practice to synchronise these events. Furthermore, the experimenter had also to operate a foot switch. This acted as an event-marker, using an electronic device connected to the Imager, which had been developed by the Department of Psychology's technical staff.

4.3.16. Immediately following this pre-stimulus period, four frames were recorded during stimulus presentation. Simultaneous with Frame 5, the stimulus container was silently uncapped and held under the subject's nose, at a distance of approximately 5 cm. This can be seen in Plate 3, above. This phase was event-marked, as described above. If the subject moved their head, the experimenter maintained the stimulus in the same relative position. At the end of frame 9, the stimulus was removed and the cap re-applied to avoid any escape of odour. Recording continued for another three frames and then the Imager was switched out of record mode. The mother could then resume normal interaction with her child whilst the experimenter checked that recording had taken place properly. This allowed at least a two-minute inter-trial interval. Due to occasional technical problems with the floppy disk drive, data were lost or corrupted. No attempt was made to repeat an odour presentation. This was due to uncertainties about the result of practice effects on data interpretation.

4.3.17. The remaining odours followed the same pattern, with the experimenter presenting as many of the 10 stimuli as the subject would tolerate. Unfortunately, a number of subjects only 'lasted' for only one or two stimuli before beginning to cry. At this point, testing was ended. Mothers were given a Polaroid instant photograph of their child in the experimental situation and a copy of one of the brain maps generated during the session. No other remuneration was offered. The electrode headcap was then removed. Any questions were answered and sometimes this broadened into a

discussion of olfaction, following which the mothers were thanked for their participation before leaving.

Results

4.3.18. Of the 26 subjects who took part in this pilot experiment, 18 (69%) were used in a pilot study aimed at developing a technique for Respiratory Plethysmography (RP) measurement of odour response. These subjects were recruited for the RP work because of poor headcap fit or subject intolerance. Due to development problems with data recording, the records from this pilot work were later found not to be amenable to analysis and were therefore discarded. The remaining eight subjects (31%) were tested on the Brain Imager. Judging from the topographical maps alone, it seemed that most activity was located in the Delta (up to 4 Hz) waveband. Some slight activity was also seen in the Theta (less than 8 Hz) band. There seemed to be distinct qualitative differences in map appearance following stimulus presentation, which usually took the form of a fairly global change in activity. None of these results received statistical corroboration, because at this time no systems had been developed to allow this.

Discussion and Conclusions

4.4.1. Four main points arose from this pilot work. The first was that the subject recruitment system worked well enough to allow volume testing of infants. It was gratifying that sufficient numbers of parents were prepared to bring their babies in for testing. No particular problems were foreseen in maintaining the level of recruitment.

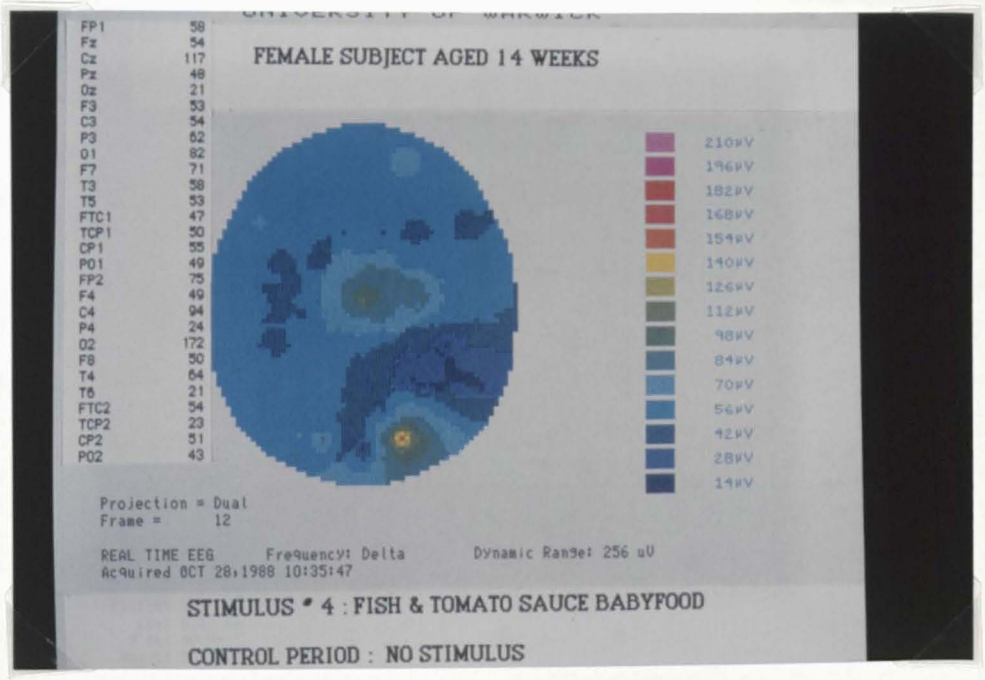
4.4.2. The second main area concerned the problem of adapting the Brain Imager for solo operation in infant testing. As described above, the technique had previously only been used for adult testing by two experimenters. However, few of the babies tested in this pilot work with the Brain Imager produced usable data. The reasons for this were believed to be related to insufficient experience with headcap fitting and the resulting problems of data contamination with movement artifact. It was felt that this would improve with practice. Nevertheless, most of the subjects seemed to

respond when presented with the odours. This usually took the form of a behavioural response whereby the subject stopped moving and focussed attention on the stimulus. This was presumed to be a form of 'orienting' response, as described by Lynn (1967) and discussed by Rosenblith & Sims-Knight (1985). The possible meaning of this response in relation to odours is discussed in the final chapter of this thesis.

4.4.3. The purely qualitative topographic maps mirrored this response, in that the map before odour presentation looked different from subsequent maps. This can be seen in Plate 4. It was hypothesised that this cortical response might be due to the visual appearance of the food stimuli. Each food stimulus was a different colour and therefore presented a different visual impression to the subjects. Because of this, it was felt that a subsequent study would need to avoid this, so it was decided to conceal the stimuli with a folded white paper tissue. This would have the advantage of being visually uninteresting to the subjects whilst not hindering the build-up or release of head-space.

4.4.4. Related to this topic was the alternative hypothesis that any response to the stimuli was simply the result of generalised 'arousal' caused by novelty. In other words, subjects would show the same kind of cortical response to any sensory experience, rather than just an olfactory one. A way of testing that was to use a non-olfactory stimulus to see whether this hypothesis had support. Accordingly, it was decided to use an auditory stimulus to see whether the cortical response could be accounted for merely by arousal. It was hypothesised that the auditory response would differ qualitatively from those produced by the odours.

4.4.5. The third lesson learned from the pilot work was that the number of stimuli was too great. Very few subjects 'lasted' through all of the 10 stimuli. In fact, most subjects only tolerated three or four before compliance disappeared. It was therefore decided to choose a smaller sample of stimuli for use in subsequent work.



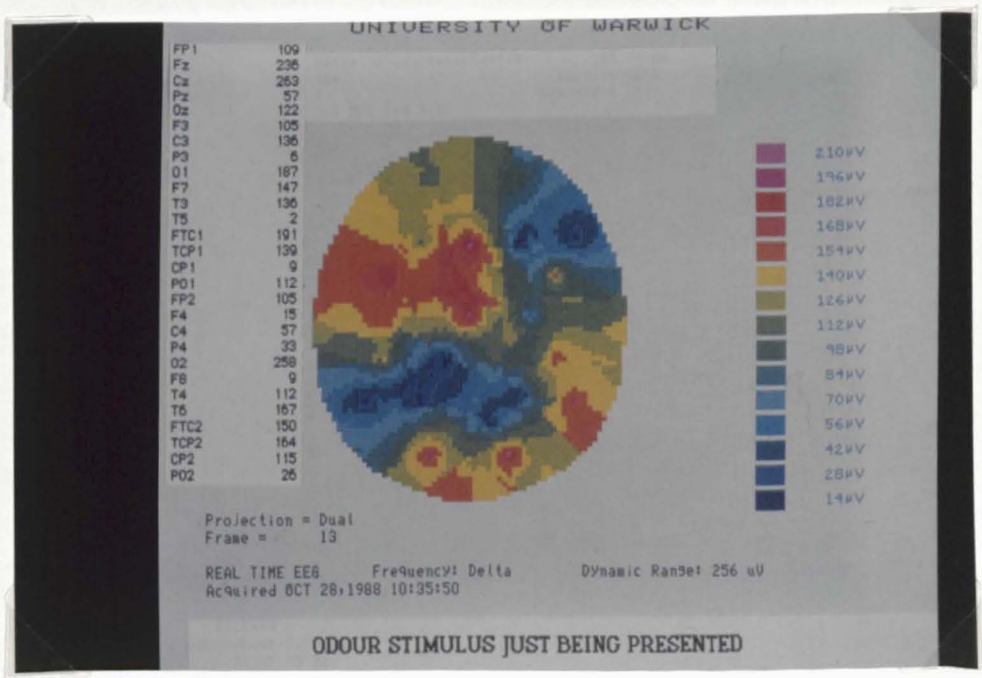


PLATE 4:

AN EXAMPLE OF THE TOPOGRAPHIC MAPS PRODUCED BY THE BRAIN IMAGER. FIGURES TO THE LEFT OF THE MAPS ARE VALUES FOR EACH ELECTRODE, IN MICROVOLTS. MAPS ARE SEQUENTIAL AND 2.56 SECONDS APART. WAVEBAND IS DELTA. NOTE CHANGES IN ACTIVITY AT ODOUR PRESENTATION.

4.4.6. The fourth lesson learned from the pilot work was that there was a need to move on from the purely qualitative dimension represented by the topographical maps. As previously mentioned, the topographical maps could not effectively be used to test hypotheses. Furthermore, the Brain Imager's systems did not permit access to the raw data that made up the maps. Methods had to be found to permit these two necessary goals. The next section discusses how developments were brought about to achieve this. This section is divided into two sub-sections. The first of these summarises two years' of development work in data handling techniques. The second section deals with the balance of strategies in deciding how to analyse BEAM data.

The data problem in BEAM

1) Data handling techniques: development

4.5.1. With the increased use of computerised measuring devices in psychology, the problem of the sheer volume of data becomes acute. With the particular system used in this study a mere 75 seconds of recording (representing one subject's data in only one condition) yielded up to 4 200 integers, in the five classic wavebands. A subject producing data in five conditions would therefore represent a set of 21 000 numbers. A system had to be developed to deal with such large data sets. This is described in detail in the Technical Appendix (Appendix 2). The system worked satisfactorily and data sets of more than 0.25 megabytes (Mb) could be handled with ease. However, the problem of how to analyse these data remained. It should be emphasised that the 'raw' data used in this analysis were not really 'raw' in the sense that they were the actual EEG data collected from subjects. In fact, as discussed in the Technical Appendix, all data had unavoidably been transformed at least three times from the real 'raw' EEG analogue data collected on the infant subjects' scalp. The first transformation involved electronic filtration and digitisation during acquisition. The second involved Fast Fourier Transform into wavebands. The third transformation involved artifact rejection. Hence the data that finally arrived for analysis were representations of the 'original'

data, rather than actual, 'raw' data. This sort of problem is not, of course, confined to EEG analysis.

4.5.2. Before analysis could commence, another area had to be examined. This was the practical problem of how to deal with the 'noise' or artifact in the data. As described elsewhere, a proportion of these data would inevitably contain artifact, from whatever source. Good headcap fitting technique, developed after much trial and error, helped to minimise this to some extent. However, the relatively high electrode impedances found in babies contributed a source of 'noise'. Furthermore, artifact caused by movement was inevitable, given the age of the subjects. This derived from motion of the head, facial muscles, eye-blink and potentials picked up from the *frontalis* muscle of the scalp. This particularly affected the frontal recording electrodes, FP1 and FP2 (see Figure 7, below).

4.5.3. In traditional EEG recording, the electrophysiologist would visually scan the paper record and locate such artifacts according to criteria learned by practice. These could then be deleted from any analysis. With the Brain Imager, a similar technique is possible. The researcher can scan the maps, and also the raw data that have been stored, looking for anomalous high areas of activity in specific areas and frequencies. These can be noted and not included in the analysis. This visual method is claimed to be superior to any computer algorithms yet devised (Rappelsberger & Petsche, 1988). At an early stage in the work described in this thesis, a decision was made not to arbitrarily delete suspect files, or parts of files before analysis. The reasoning was as follows. In adult electroencephalography, EOG and EMG contamination is often found in the Delta waveband, in the frontal electrodes. For infants, the predominant frequency seems to be the same waveband, or a little higher, as suggested by Lindsley (1939) and stated by Vos (in Dolce & Künkel, 1975), who asserted that baby EEG does not contain much information above 8 Hz. At an early stage, it was decided only to analyse data from the lowest frequency band, Delta (< 4 Hz). Experience in pilot work had led to agreement with Vos that the maximum cortical activity in babies is seen in Delta. Therefore, the chance of needlessly discarding valuable data in what was always

going to be a small sample was judged to be high. In other words, it could not be known for certain what was represented by high frontal electrode values in infants.

4.5.4. Secondly, as little is known generally about the appearance of the normal infant brain maps, it seemed inadvisable to apply adult criteria for artifact to infant EEG. Therefore, a more intuitive approach was taken. Once it became possible to download the data sets reliably, it became clear which values were anomalous. This was even more obvious when the values could be plotted graphically. By this method, 'outliers' presumed to represent movement artifact became prominent. This was notably the case with the frontal electrodes in the Delta waveband described above. Thus the problem of at least **identifying** probable artifact lessened. A further preliminary step in the data handling discarded the first 'frame' of each and every data set. This usually contained some data of doubtful provenance, due to electronic recording artifacts. With this problem tackled, possible methods of statistical analysis could be considered.

2) Analysis strategies

4.6.1. The main aim of this stage of the experimental programme was to develop an analysis technique that was not merely descriptive, but allowed hypothesis testing. As described in Appendix 2, several data analysis methods could have been used, but were subsequently discovered not to be suitable. The majority opinion amongst EEG researchers in psychology appears to be that there is no single definitive analysis technique (Rappelsberger & Petsche, 1988). Essentially, the investigator is asking deceptively simple questions of the data. What needs to be known is whether the EEG before an experimental stimulus differs significantly from that recorded after an experimental stimulus. In other words, an inferential statistical technique to lend support to the experimental hypothesis. The main thrust of the analysis strategies described in this thesis was to try and come up with a technique that adequately dealt with the data in an inferential rather than a purely descriptive way. It should be made clear that the enormous problem of analysing EEG has its own literature, which Dolce & Künkel (1975)

cover reasonably well. The main conclusion is that EEG contains so much data that a large number or combination of statistical techniques would be needed to completely characterise this plethora of information. One corollary of this is that the experimenter needs to be selective in what characteristics of the data to analyse.

4.6.2. However, this is complicated by a number of factors, as a number of assumptions have to be considered. The first of these is that the baseline or 'control' EEG activity, against which to compare the experimental condition, is both definable and relatively stable. This is an unlikely assumption, due mainly to the highly dynamic nature of EEG and the very great amount of 'resting' analogue EEG activity that is continuously present. This is what Skarda & Freeman (1987) call *"the background activity manifested in the 'spontaneous' EEG"*. This derives from any number of sources, is variable on a moment-by-moment basis and comprises a large range of frequencies and phases (Cooper, 1975).

4.6.3. The problem is, therefore, to define a meaningful summary of the 'resting' EEG, in order to compare this with the 'stimulated' EEG. One way of trying to infer a 'baseline' is by means of descriptive, summary statistics. However, this may not be a true reflection of the actual activity, because at any given instant, some values may be increasing when others are decreasing. This is part of the 'phase problem'. Any statistic involving reduction to a mean value does not reflect this accurately. This is complicated by the wide variability of the data, as surface EEG tends to be anything but homogeneous, even with the same subject. This is presumably a reflection of 'state', in terms of alertness and arousal, which may also be reflected in cortical activity. The net result is a continuously changing amount of 'background' EEG activity that makes a unitary definition of 'baseline' very difficult. This is certainly the case in adults.

4.6.4. As mentioned elsewhere, little is known about this activity in infants. Hence, the 'baseline' activity of the infant cortex has to be inferred, rather than measured directly. Because of the wide within-subject and between-subjects variability in 'baseline'

EEG, the use of a separate control condition in the experimental design becomes difficult to justify. Because individual differences appear so great in infant EEG, meaningful definitions of an average 'baseline EEG' are extremely difficult to make. Hence, it was decided at an early stage to use each subject as their own control; a within-subjects design. It was realised that this meant that the extent to which the results could be generalised was hard to define.

4.6.5. The point of the previous discussion is to highlight the fact that analysis of Brain Imager data is very problematical. The various problems presented by the data, including the difficult definition of a 'resting' or 'baseline' EEG, had to be tackled somewhat intuitively. The difficulties of phase and amplitude direction, mentioned above, deal with variables that may not be amenable to current statistical practice. Nevertheless, some exploratory analyses had to be tried out. So, as described below, this began with the most commonly-used tests. With the above discussion in mind, it can be seen that compromises necessarily surround statistical analysis of BEAM data.

EXPERIMENTAL STUDIES

Experimental Study Number 1 (BEAM)

Subjects

4.7.1. Subjects for this study were recruited by the methods described for the pilot work above. In addition, some had heard of the study from their family doctor or Health Visitor. Seventeen subjects produced complete, or semi-complete data sets for this study. Although subjects' gender was recorded, they were not subsequently classified on that basis. This was because no information was available on gender differences in the EEG of infants in this age-group. Mean subject age was 12.6 weeks (s.d. of 1.2). Obstetric and perinatal medical history was briefly recorded by asking the mother and on this basis all subjects were deemed healthy and obstetrically 'normal'. This was operationally defined as no major perinatal illness or major obstetric complication. Parents were asked about weaning status and current diet. This enabled

subjects to be subsequently classified as either predominantly breast-fed or bottle fed, and either weaned or partially weaned. However, data were not later grouped according to these criteria because of the small sample size. Mothers were also asked about time of last feed. Experience in the pilot study had shown that only those subjects who were not hungry at the time of testing stood a chance of producing usable data.

Subject selection

4.7.2. The major criterion for inclusion in the Brain Imager study concerned skull size and shape. The design of the infant-sized headcaps limited those subjects suitable for testing. Previous experience had shown that many infants, relative to adults have a skull shape that is longer in the antero-posterior axis (macrocephalic) than the lateral-lateral axis. In some subjects, this problem combined with skull distortion due to forceps or ventouse method (vacuum) birth delivery debarred them from the Imager study. Hence, the same strict selection criteria were applied as in the pilot study. Naturally, a number of subjects, even in this category, failed to produce data because of lack of compliance in the fitting and wearing of the headcap.

Apparatus and Materials

Brain Imager

4.7.3. Subjects were tested using the Neuroscience Series III Brain Imager. A new, ultra high-capacity storage device had been added to this system. This was an Optical Disk Drive, which used an recording medium similar to the Compact Disk which is used to record and play back music. Further details of this technology are included in the Technical Appendix.

Headcap fitting

4.7.4. Experience in the pilot work had emphasised the need for high-quality recording to avoid contamination by poor electrode contact. The use of the blunt needle to fill the headcap electrodes was used as before. However, electrode impedances were not measured for reasons described above.

Stimuli

4.7.5. Food odour stimuli were prepared in the same way as for the pilot study. Stimuli were as follows:

1. **Chicken Dinner**
2. **Chocolate Pudding**
3. **Beef Dinner**
4. **Fish and Tomato Sauce**
5. **90 dB tone at a distance of approximately one metre**
6. **Blank stimulus (cup containing only a folded tissue)**

4.7.6. Stimulus orders were shuffled for each subject and between subjects. The exception was the tonal stimulus (number 5). The introduction of this high-pitched stimulus was made, as discussed above, to preclude the possibility that any cortical responses seen in this experiment were due to non-specific arousal, and hence could be considered epiphenomenal. The tone was generated by an electronic oscillator and was always presented as the final stimulus. This was because it was unknown what effect the sound might have had on the subject's compliance. The loudness of the tone was provided by the manufacturer, though the frequency was not measured.

4.7.7. The food odour stimuli were chosen following experience in the pilot work described above. Their odours appeared highly consistent between manufacturer's batches, and hence reduced a likely cause of variability. Stimuli 1 and 3 appeared both perceptually similar and iso-intensive to the experimenter and other adults. Stimulus 2 was chosen because it was both acceptable to the mothers and because the odour was distinctively different from the others. Stimulus 4 appeared iso-intensive to Stimuli 1 and 3, but distinctive from either. Hence the stimulus list comprised two similar, iso-intensive odours (1 and 3), one dissimilar iso-intensive odour (Stimulus 4) and one distinctive, odour that was more intense than all the others (Stimulus 2). The babyfood was shielded from the subjects' view with a folded paper tissue. This was to lessen any visual impact from the colour of the baby food, as described above. The cup was then securely capped to allow head-space development.

Procedure

4.7.8. Parents were welcomed to the laboratory, which was as described for the pilot study. They were briefed as before on the purpose and background to the study and the sequence of events was described in as much detail as the parent required. A written consent to participation was obtained from the parent or guardian, as previously described. The same explicit offer of withdrawal was always made. Subjects were made comfortable by feeding and diaper changing if needed and seated on the mother's lap. Occasionally the father performed this support function. Ear electrodes and headcap were fitted as previously described.

4.7.9. Once the subject was judged settled by the experimenter, recording of the first stimulus began. Stimuli were presented in the same way as for the pilot work. One complete recording trial is summarised below:

Phase 1. Frames 1-4 inclusive : control period (no odour stimulation).

Phase 2. Frames 5-8 inclusive : experimental stimulus (blank or odour).

Phase 3. Frames 9-12 inclusive : control period (no odour stimulation).

Phase 4. Frames 13-16 inclusive : experimental stimulus (blank or odour).

One frame equals 2.56 seconds, which meant a possible total recording time of approximately 40 seconds. Stimuli were presented or removed as the frame counter on the Imager's display monitor changed. There was a variable inter-trial interval of not less than 30 seconds and usually at least one minute. The order of presentation of odour or blank between Phases 2 and 4 was shuffled for each subject and each trial. The aim of this was to minimise order effects that might complicate interpretation results.

4.7.10. Successive trials involved the use of different stimuli up to a maximum of six per subject. Following each successful trial, the subject was settled down again if necessary. Not all subjects received all possible odours and hence reached the final presentation which was the tone stimulus. Those subjects who completed at least one complete condition were later included in the total data set for that odour. Following completion of the testing session, the same procedure as described for the Pilot Study was followed.

Data handling

4.7.11. Prior to any numerical data manipulation, the cortical maps were closely examined for any obvious differences according to condition. Only data in the Delta waveband were examined in detail, because preliminary review of the maps had revealed little activity in other wavebands. Some subjects appeared to show marked, global increases in cortical activity in response to odour presentation. Others would show a global decrease in activity. Several subjects, as mentioned previously, had records that contained too much (>75%) movement artifact to make further analysis worthwhile. These subjects' data were discarded at this stage. Other subjects had partial but relatively artifact-free records, which were included in the total data set for that odour. In all, 17 subjects were included in the data analysis.

4.7.12. Subsequent to this initial screening of the qualitative topographic maps, the data were handled according to the following pattern:

Step 1 - All the data sets not eliminated in the initial screening were downloaded by the PtS system, described in the Technical Appendix (Appendix 2) and randomly checked to guard against loss or corruption of data during the download process. Data were then transferred to the University of Warwick mainframe computer, separated into odour type by subjects.

Step 2 - Data were read into a pre-analysis program where artifacts were discarded according to user-definable criteria. For this

analysis, the artifact limit was set at **250 microvolts.** This was a figure based on how the Brain Imager deals with very high electrode values, such as those derived from artifact. Hence, any value equal to or greater than 250 microvolts was recoded as a 'missing value' in a form that would not affect the calculation of means. Frame 0 (the very first frame recorded) was discarded entirely, because it usually contained anomalous values derived from the start of recording.

Step 3 - Data were then read into commercial analysis software (Statistical Analysis Systems, or SAS®) where a Multivariate Analysis of Variance (MANOVA) was done which addressed the following factors in the Delta waveband only:

SUBJECT = subject identifier code number
GROUP = 1 (averages of frames 1-4), 2 (averages of frames 5-8), 3 (averages of frames 9-12) and 4 (averages of frames 13-16).
STIMULUS = odour stimulus identifier code
CONDITION = 1 (averages of frames 1-4), 2 (averages of frames where blank stimulus was being presented), 3 (averages of frames 9-12) and 4 (averages of frames where odorous stimulus was being presented)
ORDER = 1 (if the blank stimulus was presented during frames 5-8) or 2 (if the blank stimulus was presented during frames 13-16).

It will be seen that the GROUP and CONDITION factors were very similar and were defined as an aid to clarifying any order effects.

4.7.13. It was thought prudent to use MANOVA, rather than ANOVA because of the 'sphericity assumption'. Consideration of the 'sphericity assumption' (Vasey & Thayer, 1987) is particularly important within the sort of 'repeated measures' designs commonly used in psychophysiology. This assumption concerns the distribution of variance of all the contrasts in a repeated measures design, which can seriously affect the validity of some inferential statistical tests. This is particularly so in psychophysiology studies where data measurements may be highly correlated both spatially and temporally. As Vasey & Thayer put it:

> *"In general, the p values of the F tests are accurate only when the variance-covariance matrix* Σ *can be said to be circular. This is true if and only if the variance of all the contrasts between repeated measurements which compose the overall comparison of interest (e.g. the within subject main effect) is constant "* (page 480).

These authors recommend the use of multivariate tests, which do not assume sphericity and make no inflexible assumptions about the normality of the distribution of the data. However, a larger sample size is required in MANOVA than repeated-measures ANOVA to attain reasonable power. Hence, the decision about the type of parametric test was a compromise between sample size and likelihood of sphericity assumption violation. For an exploratory analysis, MANOVA was deemed suitable. All possible combinations of factors were produced by the program.

Results

4.7.14. The results of this analysis were as follows. Only two of the factors or interactions were significant. These were **SUBJECT**: $F = 24.49$, d.f. = 15, 176, $p < 0.001$, and **STIMULUS** (stimulus type): $F = 22.59$, d.f. = 3, 188, $p < 0.001$.

Discussion and Conclusions

4.7.15. The main conclusion was that the BEAM technique was usable as a method of investigating infant sensory function. The modified paradigm used in Study One was a partial success, showing that stimulus and cortical response could be linked. In other words, the use of corroborative event-marking and precise timing of response presentation made this possible.

4.7.16. The other main conclusion also concerned the stimulus-response link. If cortical activity were random and unconnected with odour presentation, then presumably no difference between odours would have been demonstrated. It was clear from close examination of the cortical maps that changes in activity were closely linked with experimental manipulations. The statistical analysis tended to corroborate this admittedly subjective interpretation,

though not in a conclusive fashion, because of a more tenable alternative hypothesis described below.

4.7.17. In fact, the MANOVA results were somewhat puzzling. Why, if there were a significant between-odours difference, was this not reflected in differences with the other factors included in the analysis? In other words, it made no difference what was happening to the subject, in terms of sensory (odour) stimulation, during each trial. Furthermore, the lack of any interaction between condition and stimulus suggested that any response was as pronounced during a 'blank' stimulus as an odorous one. This is discussed below.

4.7.18. The corollary of the above is that some subjects, who were receiving the odorous stimulus early in the trial, had their cortical activity boosted to a higher level than those who received the odorous stimulus late in the trial. This might have simulated a between-odours difference where none actually existed. However, this implies that some confounding effect was also in operation. In adults, this would suggest that the subject 'knew' that an odour was to be presented early in the trial. This explanation is unlikely in infants. The way to avoid this problem would have been to modify the method so that the interval between experimental stimulations (either odorous or blank presentations) was at least as long as the inter-trial interval. This presented practical problems to do with subject compliance, as many babies became restless during recording and the amount of artifact increased as a function of recording time.

4.7.19. Another possible interpretation is that the data had been excessively 'smoothed' by the artifact-rejection process inherent in Step 4 above. This process might have somehow removed subtle differences in cortical response between the 'no odour' condition and the 'odour presentation' condition. However, repeating the analysis at a later stage, using more lenient or even non-existent artifact criteria produced essentially comparable results. Hence it seemed unlikely that excessive 'smoothing' of the data was the reason. The result with the **SUBJECT** factor may have been accounted for by the wide between-subject variability of data.

4.7.20. An alternative analysis strategy would have been to consider each electrode location separately for each frame in subject. However, using 'electrode' as an independent variable with 28 levels would have lead to an excessively high number of degrees of freedom in an Analysis of Variance. With such a high number, spuriously significant results would probably have emerged and led to concerns over violations of assumptions, as described above. Furthermore, this would have led to a much larger sample needing to be tested to compensate for this.

4.7.21. Another explanation for the general significant difference between odours was that some kind of 'carry-over' effect was operating. It is possible that, given the overall short time of the trials, subjects were failing to 'recover' their previous baseline EEG activity before another stimulus was presented. Some subjects showed a change in EEG activity with the very first stimulus and this did not diminish in the time available. In other words, a kind of very prolonged 'alerting' response was occurring. The subjects' 'baseline' EEG level was shifted by the presentation of any kind of stimulus, and this failed to decay during recording. This suggests considerable novelty associated with the stimulus. This accounts for the lack of significant differences within each trial when there was an overall difference between trials in the between-subjects sense.

4.7.22. Most subjects showed a clear behavioural response when presented with the odour container. They fixated their attention upon it and ceased moving for a few seconds in a classic orienting response (Lynn, 1967) and then resumed activity. As suggested above, it is also possible that this brief response was more persistent in terms of cortical activity and influenced subsequent recording throughout the trial. It is also possible that some of the odorous stimuli (perhaps the perceptually 'stronger' ones) were more effective than others in causing this orienting response. These stimuli provoked more fixation and less movement. This resulted in less movement artifact being recorded. A way to test this would have been the use of simultaneous *frontalis* EMG recording. The hypothesis would have been that the 'stronger' the stimulus was, the less movement artifact would have been recorded. However, the

practical problems of this rather invasive technique are formidable. It would been extremely difficult to devise a safe transducer and fixation method to record peri-orbital EMG from babies. Not only that, but the Brain Imager could not have been adapted to record such information.

4.7.23. The most plausible alternative hypothesis that might explain these findings is that subjects were responding not only to the odorous stimulus, but also to other perceptual cues. This is explanation for the results that was accepted by the experimenter. During testing, the mother was requested not to interact with her baby and this advice was usually followed. Nonetheless, the mother might have unconsciously cued her infant by involuntary movement when the odour stimulus was presented. Furthermore, some visual cuing of the subjects by the actual presentation of the stimulus container was unavoidable, as a visual stimulus was being presented simultaneously with an olfactory one. It is widely accepted that infants, of the age tested in this experiment, are visually dominant (Werner & Lipsitt, 1981). In other words, the visual component of the subjects' response to the odour overshadowed the olfactory response. The testing situation may have had high novelty salience to infants, despite the precaution of screening the contents from view with a folded tissue. Interestingly, a number of subjects displayed masticatory movements when presented with the stimulus container. Hence, the olfactory stimulus had a visual component, which was a confounding variable. This was the final interpretation of the results of this first study. Consideration of all these points led to a redesign of the experimental technique, leading to Experimental Study Two. The main purpose of this study was to minimise visual cuing. Before this is described, an important conceptual point needs to be made.

4.7.24. This concerns the relationship between areas of cortical activity and their underlying anatomical correlates in infants. This is discussed in Blume, Buza & Okazaki, (1974) and also in Hellstrom, Karlsson & Müssbichler, (1963). These latter workers concluded that the relationship between surface electrode placement and areas of the underlying brain is hard to define. These studies argued for a fairly fixed electrode/structure relationship in the subjects tested,

though they make it clear at the outset that this view may be debatable by saying:

> *"... in these small children the relation between the external landmarks of the skull and the positions of the underlying brain differs from that in the older age group. Apart from this, the looseness of the scalp in infants makes it still more difficult to establish topographical relationships".*

4.7.25. Blume and his colleagues (*op.cit.*, 1974) took this a stage further and cast more doubt on a consistent, between-subjects relationship between electrode placement and underlying cortical structures. They conclude that there is definite variability in this relationship, not merely between infants, but even between hemispheres of the same brain. An interpretation of this is that most infant brains do not fit into their skulls with any degree of accuracy as regards external landmark/internal structure relationships. In terms of clinical need to accurately localise lesions, for example when neurosurgical procedures are planned, this is something of a problem. However, for the type of research described in this thesis, the problem is of less importance.

4.7.26. The reasons for this are as follows. Most workers agree that EEG only reflects activity in the surface 1 mm. or so of the cortex. If it is assumed that olfactory processing occurs in a similar fashion to that of the adult, then much of the olfactory structures and processes involved are deep within the brain. This suggests that the sources accounting for surface EEG in response to odours are similarly located. Hence, it hardly matters that the correlation between surface electrode position and cortical landmarks is relatively low. In this circumstance, it seems hardly appropriate to speak of 'localisation' of response for human infants. This topic will be discussed in more detail in the final chapter.

4.7.27. To summarise this first experiment, it was shown that the BEAM technique could be used to test small infants in reasonable numbers. The system for subject recruitment worked well and the mothers were happy for their children to be tested. Probably due to

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the comprehensive nature of the pre-testing briefing, very few mothers expressed any overt anxiety about the test situation. Furthermore, the results of the first study suggested improvements that might lead to a useful analysis technique and less ambiguity in the interpretation of the results.

4.7.28. One of the main reasons for initially deciding to test subjects in an 'armchair' situation was to provide a comfortable and friendly environment. This was to try and ensure a relaxed mother and baby and improve the chances of successful testing. However, the disadvantage of this situation was that the babies were visually distracted by the odour presentation. The dominance of the visual system in babies of this age was, with hindsight, under-estimated. The interpretation given to the statistical analysis of the data tended to confirm this, as discussed above. An important conclusion, therefore, was that this visual aspect of the odour stimulus needed to be greatly reduced. In this way, cortical response could be more confidently linked to odour stimulation. For this reason, a second odour study was designed.

Experimental Study Number 2 (BEAM)

Introduction

4.8.1. The main aim of this study was to improve the experimental environment such that the possibility of visually cuing the infant subjects would be reduced as far as possible. This would have the effect of increasing experimental power by controlling an extraneous, confounding variable. The olfaction laboratory was already equipped with a chamber which was adapted for infant testing as a result of the first BEAM study.

4.8.2. Remote, silent presentation of the odours was seen as a way of minimising the external cuing described above. Hence considerable development work had to go into designing and constructing the odour delivery system which is described below. Furthermore, the Brain Imager required some additional development to allow remote event-marking of odour presentation.

Method and Equipment

Brain Imager

4.8.3. All subjects were tested using a Neuroscience Series III Brain Imager, as described earlier. Settings were identical with previous studies.

Low Odour Room (LOR)

4.8.4. Infants were tested whilst seated on the mother's lap, in a low ambient odour chamber (see Figure 3 and Plate 5). This chamber was designed to minimise external cues available to the subject by being both quiet and dimly-lit. Low ambient odour within the LOR was maintained by the following construction and maintenance regime:

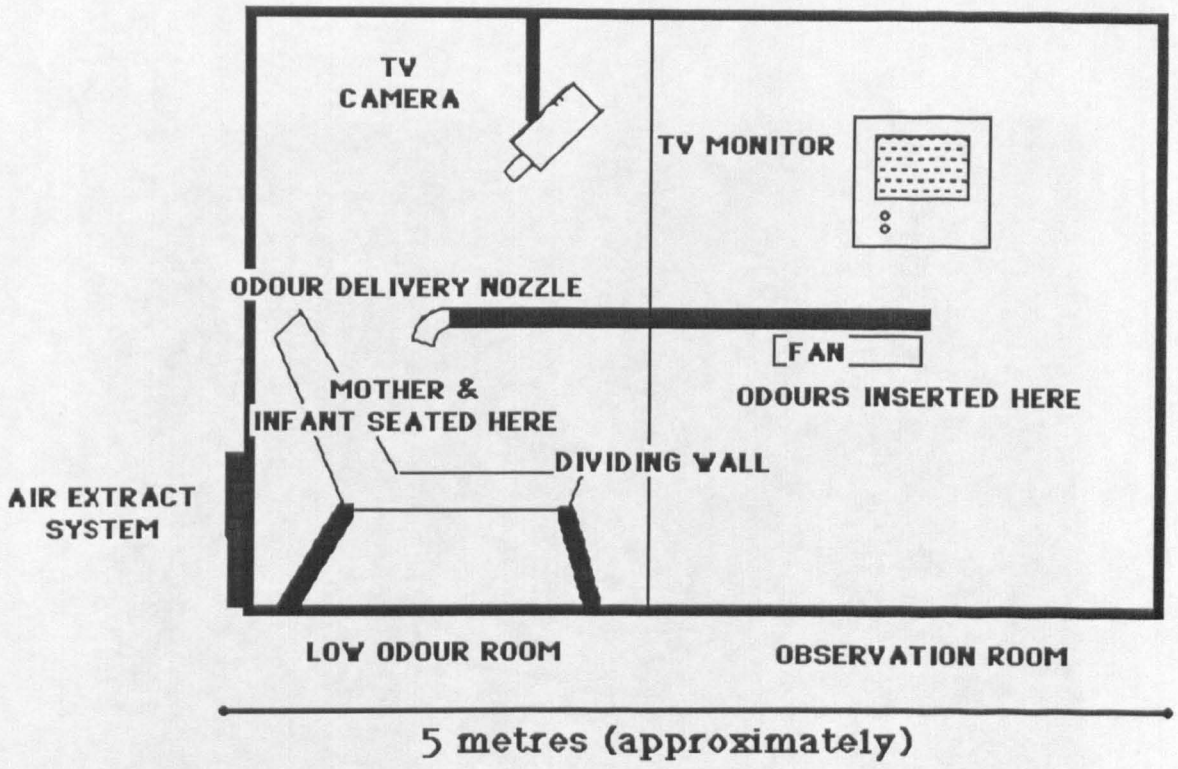
1) Anodised aluminium wall and ceiling linings.
2) Continuous use of ventilation system; regular filter changes.
3) Special ceramic tile construction of floor, sealed with polyurethane sealing compound to minimise odour from tile grouting.
4) Strictly no use of non-experimental odorous material within the chamber.
5) Regular 48-hour application to floor of granulated magnesium silicate (Steetley Minerals Limited) to absorb any residual odours.

LEGEND TO PLATE 5:

THIS PICTURE WAS TAKEN FROM THE OBSERVATION ROOM. THE VIDEO CAMERA FOR OBSERVING MOTHER AND SUBJECT CAN BE SEEN AT THE TOP OF THE PICTURE. THE ORIFICE OF THE ODOUR DELIVERY SYSTEM IS VISIBLE IN THE CENTRE AS A BLACK TUBE. BEHIND IT IS THE PRE-AMPLIFIER FOR THE BRAIN IMAGER. ALONG THE BACK OF THE CHAIR (LEFT TO RIGHT) ARE: THE HEADPHONES AND MICROPHONE WORN BY THE MOTHER; THE SENSOR FOR RESPIRATORY PLETHYSMOGRAPHY; THE INFANT ELECTRODE HEADCAP.

PLATE 5:
THE LOW ODOUR ROOM (LOR)

FIGURE 3: THE LOW ODOUR ROOM (LOR)
(NOT DRAWN TO SCALE)



Mean temperature inside the LOR was 20 degrees Celsius. The LOR was fitted with a virtually silent, high-capacity ventilation system which provided 18 complete air changes/hour. Dimensions of the LOR were; 2.2 metres high; 1.8 metres wide and 1.6 metres deep. The internal volume was therefore 6.7 cubic metres. In order that the odours presented to the subjects could be remotely presented, avoiding cuing, an odour delivery system was specially designed.

Odour delivery system

4.8.5. The same type of plastic cups containing the baby foods described in earlier work were used for this study. These were positioned in a fan-assisted odour delivery system, (see Plate 6 and Figure 4) which ensured that the odour from these foods was rapidly entrained to the subjects' facial area, with a rapid rise-time. This rise-time was estimated at less than one second. This speed was

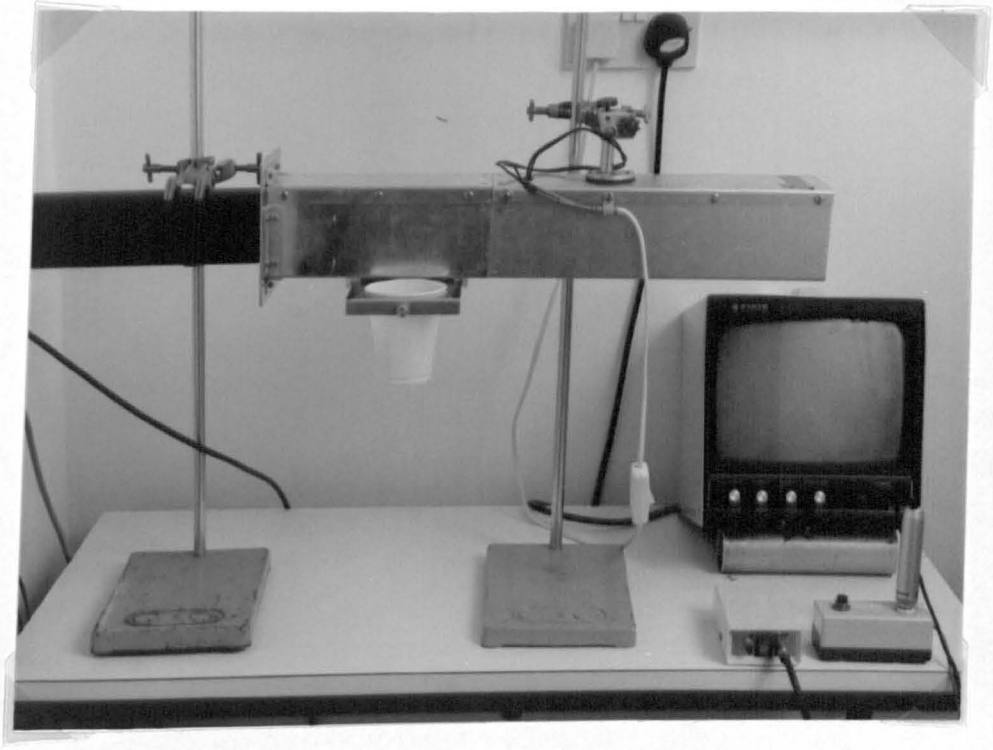
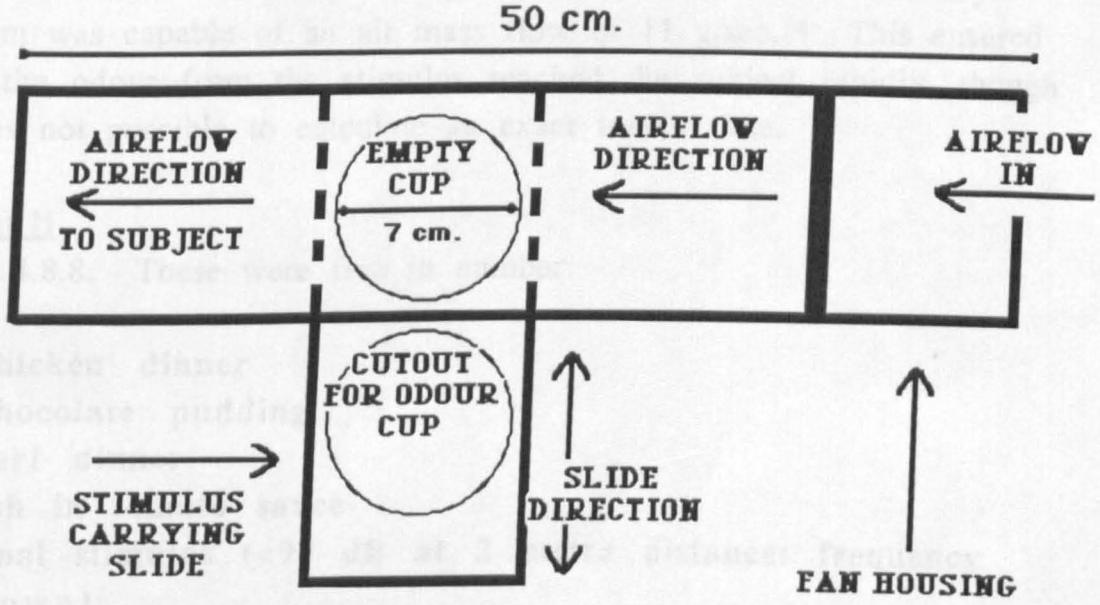


PLATE 6:

THE ODOUR DELIVERY SYSTEM. THE TV MONITOR IN THE RIGHT OF THE PICTURE WAS USED TO OBSERVE SUBJECT AND PARENT IN THE LOW ODOUR ROOM. CONTROLS IN FRONT OF THE MONITOR WERE FOR AN INTERCOM SYSTEM, AND EVENT-MARKING. ODOUR FROM THE STIMULUS CONTAINER (CENTRE OF PICTURE) ENTERED THE LOW ODOUR ROOM VIA THE BLACK TUBE (TOP LEFT OF PICTURE).

necessary so that any cortical response seen in the 2.56 second-long frame could be plausibly linked to the odour presentation. The odour delivery system was also designed to produce extremely little operating sound, and be simple to operate. The stimulus container was placed in the cutout on the stimulus carrying slide (see Figure 4). Just prior to stimulus presentation, the cap was removed and the carrying slide operated. The cup containing the baby food would thus be placed in the airstream of the delivery system and the odour head-space delivered to the subject. With practice, the carrying slide could be silently operated in under a second.

FIGURE 4: ODOUR DELIVERY SYSTEM (PLAN VIEW)



4.8.6. Air velocity at the subject's face would typically be 1 metre/sec. at the mean testing distance of 50 cm from the tube orifice inside the LOR (see Figure 3) and the airflow was slightly turbulent. An empty, uncapped cup acted as a 'control', in that the airflow through the odour delivery system passed over it in the recording period before odour presentation. This was necessary to balance the airflow during odour presentation when the stimulus carrying slide was operated. Had this not been used, a pressure change in the system would have been caused. This would have

resulted in a momentary change in airflow velocity, detectable as a 'switching pulse'. This would have been noticeable enough to cue the subjects to stimulus presentation and caused a possible confound. It is worth re-emphasising that one of the most important aims of this experiment was to avoid the cuing of subjects which had been seen in earlier work.

4.8.7. Airflow measurements of the odour delivery system were made using a hot-wire anemometer. Use of this sensitive device revealed air velocity changes at the point of odour insertion into the delivery system, due to vortices in the airflow. This meant that the turbulence associated with these vortices caused the odour head-space from the food stimuli to be thoroughly and rapidly mixed into the airstream on stimulus presentation. The odour delivery system was capable of an air mass flow of 11 g/sec.[14] This ensured that the odour from the stimulus reached the subject rapidly, though it was not possible to calculate an exact transit time.

Stimuli

4.8.8. These were five in number:

1) chicken dinner
2) chocolate pudding
3) beef dinner
4) fish in tomato sauce
5) tonal stimulus (<90 dB at 2 metre distance: frequency unknown)

The first four were identical with those used in previous experiments and were prepared in the same way. However, there was no need to screen the contents with tissue paper as they would not be seen by the subjects. The tonal stimulus was used for the same reason as the previous study and produced by the same electronic oscillator. The sound level from this device was attenuated to avoid startling the subjects when it was operated in the confines of the LOR.

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[14]This was calculated following discussions with the Department of Engineering, University of Warwick.

4.8.9. Towards the end of this study a further stimulus was introduced. This followed discussions with Professor Steiner of the Hebrew University. The reason for its introduction was to test the hypothesis that a perceptually intense stimulus (analogous to, but not as powerful as a trigeminal stimulant) might produce a correspondingly dramatic cortical response. As it later turned, insufficient numbers of subjects were tested with this stimulus to be able to test the hypothesis. This stimulus comprised approximately 50 g. of strong cheese, obtained from a supermarket. This was kept refrigerated in a sealed container between testing sessions, and renewed regularly. Prior to testing, it was allowed to reach room temperature to permit head-space build-up. It should be noted that this cheese odour was not iso-intensive with the other odours.

4.8.10. Odour stimuli were presented in a sequence that was shuffled between subjects, to minimise order effects. The exception to this was the cheese odour (Stimulus 6), which was always presented penultimately. The reason for this was that most mothers found it aversive and it was feared that they might unconsciously cue their baby as a result. The tone stimulus (Stimulus 5) was always presented last, for the reasons described in the previous study.

4.8.11. An empty stimulus container cup (as 'stimulus 7') was used as an actual control stimulus in one subject only, in that it was presented in lieu of an odour. This acted as check that no cortical response was due solely to the operation of the odour delivery system. Later inspection of the resulting maps lent strong support for the view that no response resembling that from due to an odour was seen in this condition..

Subjects

4.8.12. All subjects were recruited in the same way as in earlier studies and it was ensured that subjects were healthy at the time of testing, as previously described. Exclusions would have been limited to prematurity of more than four weeks and respiratory distress during labour or in the perinatal period, though no exclusions were made on these criteria. The sample comprised 11

subjects, 7 males, and 4 females, mean age 12 weeks (s.d. 1). No categorisations were later made on the basis of gender, for the reasons previously discussed.

Procedure

4.8.13. The experiment was explained in detail to the mother, who gave written consent. Details of the baby, regarding weaning status, mother's basic obstetric history and time of last feed were recorded, as previously described in case they were required to assist later data analysis. Trial fitting of an Imager headcap then took place. If one of the available headcaps was not a very close fit, or unacceptable to the baby the procedure was abandoned. Subjects in this category were usually used in the parallel study (Experimental Study 3) involving respiration measurement. This is described later in this chapter.

4.8.14. If one of the headcaps was suitable, the subject's head was wiped with 95% ethanol and allowed to dry, as before. Following experience with the previous study, a new technique of electrode preparation was introduced. Prior to electrode filling, the tiny area of scalp beneath the electrodes was very lightly abraded with a blunt plastic probe. The tip of this probe was convex and only protruded 1 mm. beneath the electrodes, for additional safety. Great care was taken to avoid those electrodes close to the fontanelles. This probe was tipped with OmniPrep, which is a mildly abrasive skin preparation paste. It was used to try and reduce the high skin impedances found with infant subjects in EEG work. However, it became clear during the testing programme that high impedances were usually found despite all efforts.

4.8.15. Following headcap fitting, mother and infant would move into the Low Odour Room (LOR). The infant would be seated on the mother's lap, supported comfortably in the airflow from the odour delivery system. The baby's electrode headcap was plugged into the pre-amplifier and a check for satisfactory EEG waveforms made from the Brain Imager. On several occasions, this revealed poor electrode contact which necessitated re-filling of electrodes. The mother was then fitted with headphones and a lapel microphone.

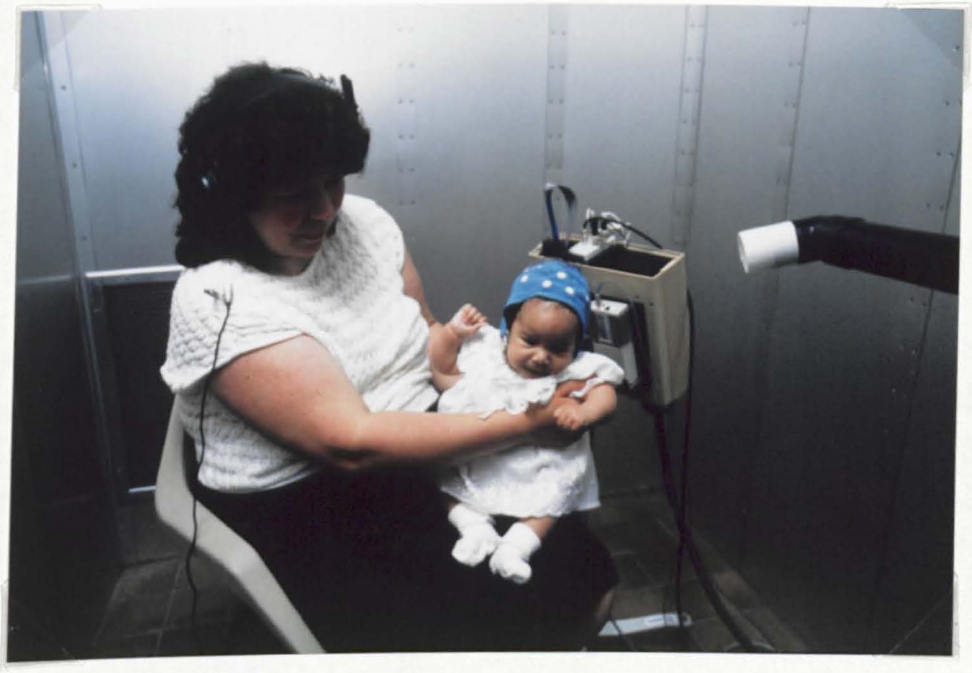


PLATE 7:

MOTHER AND BABY IN THE LOW ODOUR ROOM. THE MOTHER IS WEARING THE HEADPHONES AND MICROPHONE FOR THE INTERCOM SYSTEM. IN THIS PHOTOGRAPH, THE BABY'S ELECTRODE HEADCAP HAS SLID SOMEWHAT POSTERIORLY. THE WHITE PAPER GUARD ON THE TIP OF THE ODOUR-DELIVERY TUBE (RIGHT OF PICTURE) WAS REMOVED PRIOR TO TESTING.

The intercom system, permitting inter-trial communication between mother and experimenter, was then demonstrated. It was also explained to the mother that a low-light video camera allowed the experimenter to observe the subject at all times during testing.

4.8.16. Mothers were asked to avoid any movement, or any social interaction with their child. The mother was told, via the intercom headphones that an odour would be noticeable, but not when this would occur. This was in order to minimise any inadvertent cuing of the baby by the mother. The lights in the LOR were then dimmed to the absolute minimum level consistent with allowing the video camera to operate. This sharply reduced the amount of illumination available to the subject, though the actual light level was not objectively measured. The door to the LOR was then closed. The testing situation can be seen in Plate 7. It should be noted that the light level shown in this illustration is not representative of the actual testing situation.

4.8.17. Stimulus presentation commenced when the subject was judged settled. This was assessed by the experimenter using the video camera to observe the subject and by viewing the EEG waveforms. Stable, interference-free waveforms generally indicated a settled, relaxed subject. However, a pacifier was permitted if this was the only way that the subject would cooperate. The warm, dark and quiet testing environment usually resulted in subjects lapsing into a state of somnolence during recording. As before, no formal assessment of 'state' was attempted. Each recording session consisted of the following pattern:

Frames 0-9 :- recording started; no odour stimulation, pure airflow only.

Frames 10-20 :- odour inserted into the odour delivery system at the beginning of Frame 10, as defined by the frame counter on the Imager's monitor display. The odour was removed at the start of Frame 20. The electronic event-marker was activated on presentation and removal of stimuli.

Frames 21-30 : - no odour stimulation, pure airflow only. Recording ended.

4.8.18. This stimulus presentation pattern was repeated using as many stimuli as the subject would tolerate. As had been seen with earlier BEAM work, very few subjects tolerated presentation of all stimuli. If the subject became at all upset during the recording session, an attempt was asked of the mother to pacify her child. If this was not rapidly effective, testing was halted. Those subjects who got as far as the tonal stimulus (Stimulus 5) heard the tone between Frames 10 and 20, as above. The odour delivery system was left running throughout recording for this stimulus.

4.8.19. A minimum of 1 minute inter-stimulus interval was always given between recordings; this time was often longer in practice. During this time, the experimenter checked that the data had been correctly recorded onto the Optical Storage Disk. On several occasions, this failed to operate properly and the data were lost or corrupted. If this occurred, the odour presentation was not repeated because of uncertainties about the possibility of practice effects on cortical activity. This inter-stimulus interval had the added advantage of allowing the baby to settle down between stimuli, and the efficiency of the LOR ventilation system ensured that no odour mixtures were present in the atmosphere. If the interval between recording lasted more than three minutes, as was often the case, there would have been one complete air change in the LOR.

4.8.20. Following the recording session, the mother was debriefed as described before and given the usual souvenirs of photograph and brain map. Following data collection, the problem of data handling and analysis arose. As discussed earlier in this chapter, some progress had been with this for Study 1. In parallel with data collection, considerable development of data manipulation techniques had been continuing. The following sections describes this data handling and analysis process.

Data Handling

4.9.1. All data files from each subject received a preliminary review and inspection, as described in Study 1. Subject files that were grossly contaminated with movement artifact were discarded during this initial review phase of data handling. Three subjects' data sets were completely discarded at this point, leaving **eight subjects** having produced usable data in this study. It should be emphasised that the criteria for inclusion in the final data set were extremely rigorous. Because this experimental programme was concerned with a new testing technique, it was felt necessary to be highly conservative and minimise every possible chance of a Type I statistical error. It should also be made clear that only the Delta (up to 4 Hz) waveband was addressed, as before.

4.9.2. The next stage in data-handling was a frame-by-frame analysis of the topographic maps, looking for artifact. This allowed for its later identification and elimination from the data sets. Artifact values were operationalised according to the following criteria:

1) consistently and/or anomalously high values, as seen from the graphics program output. These were usually produced by poor electrode contact or very high impedance.

2) consistently and/or anomalously low (< 10 microvolts) values that did not alter throughout the sequence of frames. This was usually caused by electrodes that were unresponsive due to poor contact.

Artifact cut-off was set at 500 microvolts. All data points due to artifact in each file could be converted at a later stage, by the mainframe computer into special character codes. These permitted commercial analysis software to treat them as 'missing values' that would not play a direct part in the analysis. All the remaining data, as amplitude values, were then downloaded entire from the Brain Imager to a micro-computer and to the mainframe computer, as previously described.

4.9.3. Once stored on the mainframe computer, data underwent a series of transformations. The first of these involved data reduction. Because only the Delta waveband was to be analysed, data were subjected to a simple program that deleted all other wavebands. Hence each data set was systematically reduced by four-fifths. Data were then manipulated in the computer, in order that they could be read into graphics software, but without altering the data values themselves. This data handling method had been developed by the experimenter and written by the Department of Computing Services, University of Warwick. The software presented the data sets in a graphical format, so that any 'outlier' values due to artifact could be more easily identified. An example is given in Figure 5. Further examples are given in the Technical Appendix (Appendix 2).

4.9.4. Each file was then divided into two, at the junction of the 'pre-stimulus' and 'during stimulus' conditions. The frames for these were 1-9 and 10-20 respectively. The reason for this was so that a repeated-measures ANOVA could be performed. This is described below. The data flow from acquisition of EEG from the subject to final analysis is summarised in Figure 6 below:

FIGURE 5:

GRAPHIC REPRESENTATION OF BRAIN IMAGER DATA. THE ABSCISSA IS TIME IN 'FRAMES' (ONE FRAME = 2.56 SECONDS). THE ORDINATE IS AMPLITUDE IN MICROVOLTS. EACH CURVE REPRESENTS OUTPUT FROM ONE ELECTRODE. DATA ARE FROM ONE SUBJECT, ODOUR 2 (CHOCOLATE PUDDING). THE ODOUR IS PRESENTED AT FRAMES 9/10. SYNCHRONISATION OF EEG DATA CAN BE SEEN.

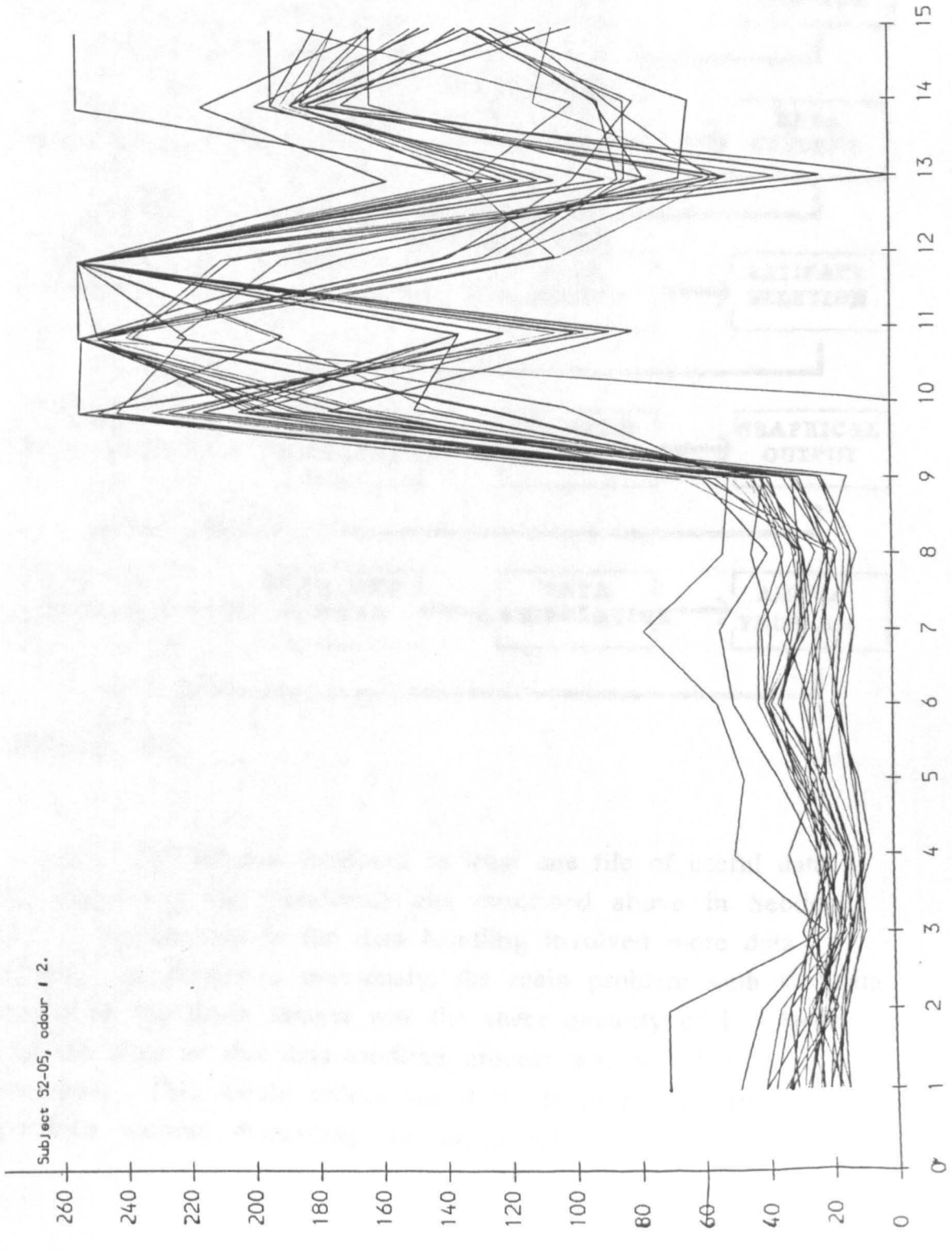
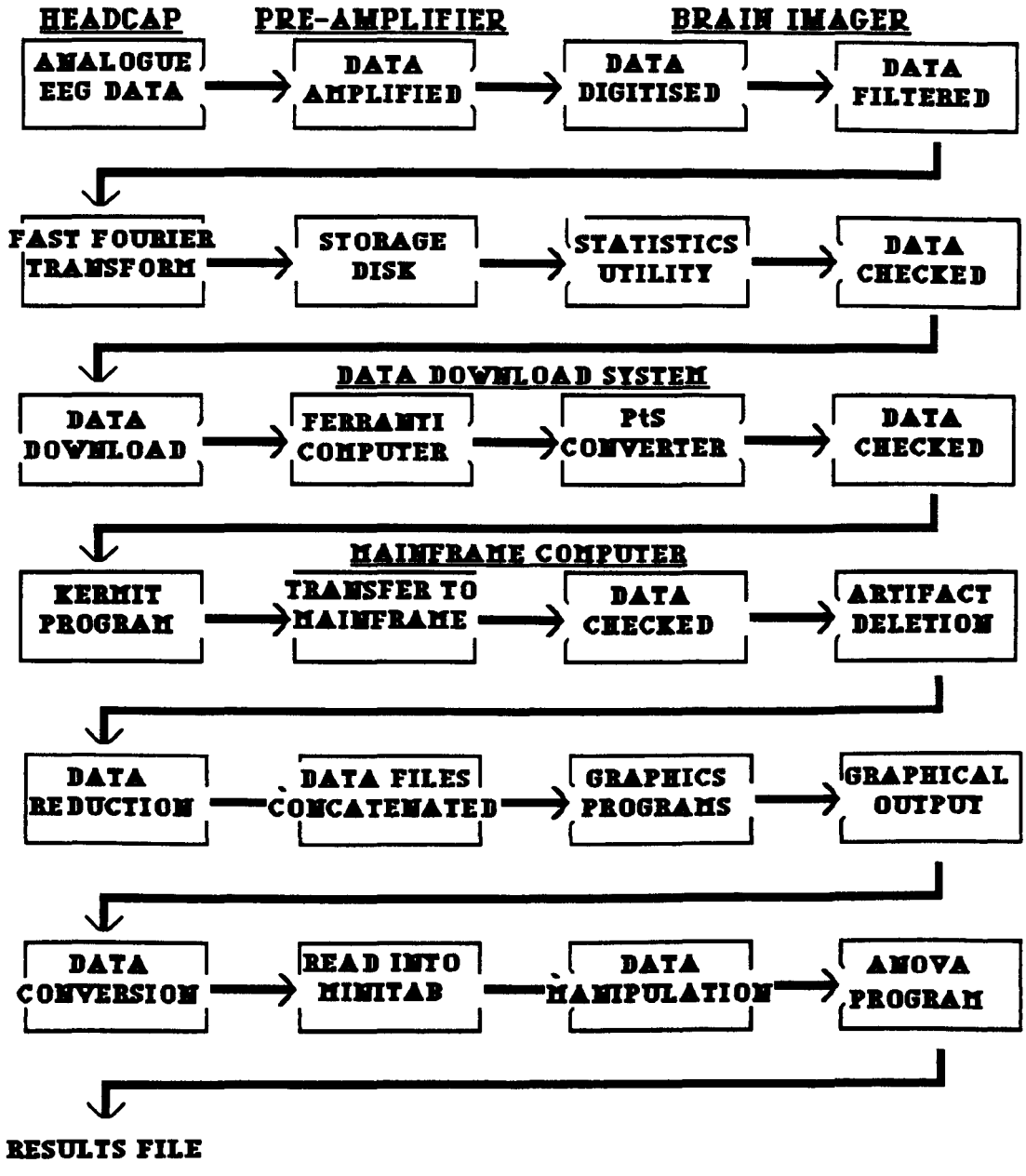


FIGURE 6: DATA FLOW CHART THROUGH IMAGER SYSTEMS



4.9.5. All subjects produced at least one file of useful data, which underwent the transformations described above in Section 4.9.3. A further step in the data handling involved more data reduction. As described previously, the main problem with the data produced by the Brain Imager was the sheer quantity of it. Hence one of the aims of this data-handling process was to effect a compromise. This would reduce the data set to manageable proportions without discarding too much information.

4.9.6. Because of this, only frames 5 to 16, of the now artifact-free data set were analysed. The idea behind this was to get a representative sample of data in the 'baseline' period (frames 1 - 9), and in the 'stimulus' period (frames 10 - 20). In other words, the data would be further summarised in the interests of clarity into 'before odour' and 'during odour'. Even so, the data-handling software produced a large number of values. There was one value for each of 28 electrodes, in each stimulus condition, for all subjects who produced data in that condition.

4.9.7. To analyse each of 28 electrodes electrode for all subjects would have produced an unrealistically high number of degrees of freedom in a subjects-by-odours ANOVA. A similar problem is discussed by Rappelsberger & Petsche (1988). A way round this was to perform separate Analyses of Variance for a subset of electrodes. Hence it was necessary to choose specific electrodes for analysis. The problem was then to choose which electrodes to include and which to discard.

4.9.8. As described elsewhere in this thesis, the frontal electrodes were a likely source of artifact and were hence discarded. There is apparently no previous infant research that could have further assisted in this choice, though a recent study using adults and the BEAM technique gave some powerful clues (Van Toller *et al*, 1990). This pioneering study, which involved a variety of odorants, found that a limited number of electrodes on the vertex of the skull were the most reliable indicators of cortical activity linked to odour perception. Hence, a similar group of electrodes were chosen for analysis of the infant data. It should be pointed out that data in the adult study had been analysed using Multi-Dimensional Scaling (MDS). This is a mainly descriptive technique unsuitable for the infant data, because the small sample size would have invalidated it.

4.9.9. The electrode subset chosen for analysis from the infant data comprised: Cz, Pz, C3, P3, TCP1, CP1, C4, P4, TCP2 and CP2. These can be seen in Figure 8. Figure 7 shows the total array of 28 electrodes for comparison purposes. The electrodes in the subset

were also given numerical labels for the purpose of analysis: Cz=1, Pz=2, C3=3, P3=4, TCP1=5, CP1=6, C4=7, P4=8, TCP2=9 and CP2=10. The reasons for choosing these particular electrodes are given in the following paragraph.

FIGURE 7: LOCATIONS OF ELECTRODES ON INFANT HEADCAPS (MODIFIED FROM INTERNATIONAL 10/20 SYSTEM)

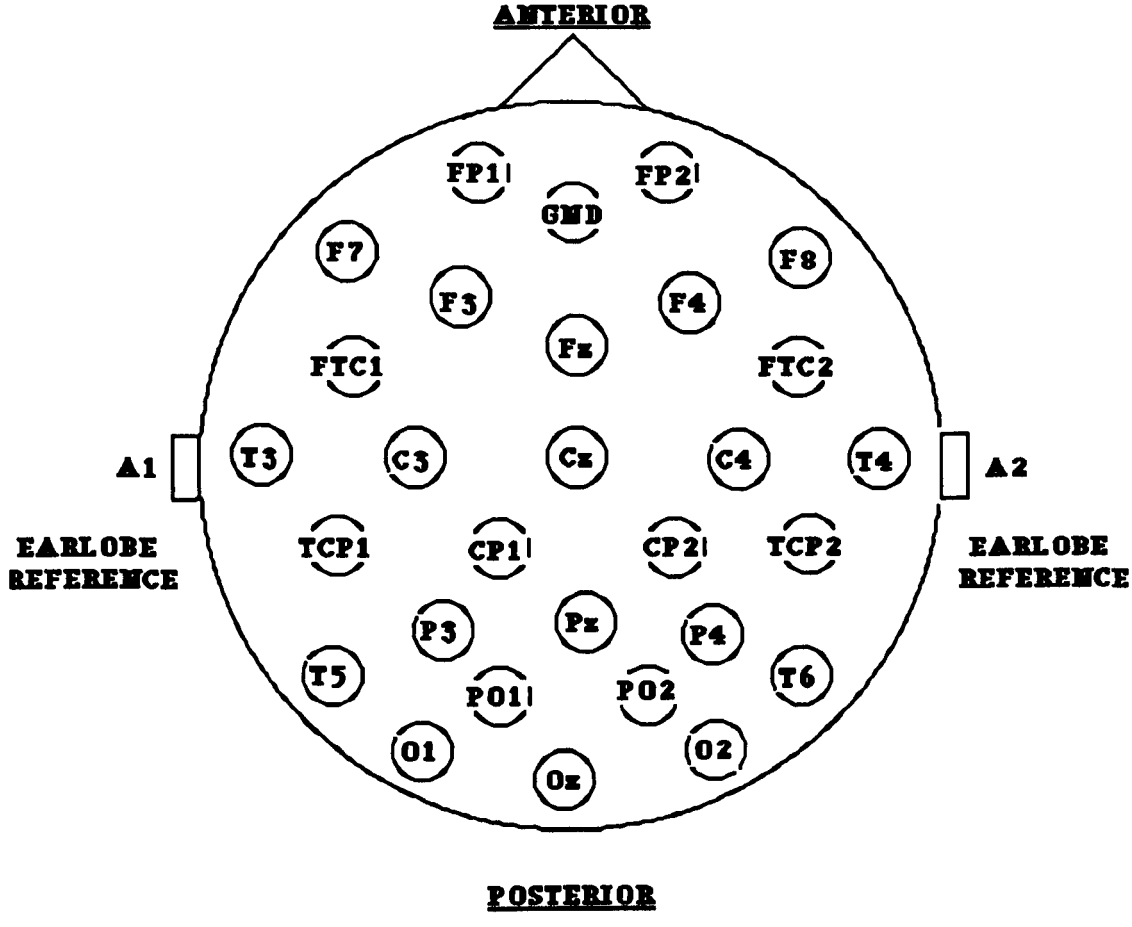
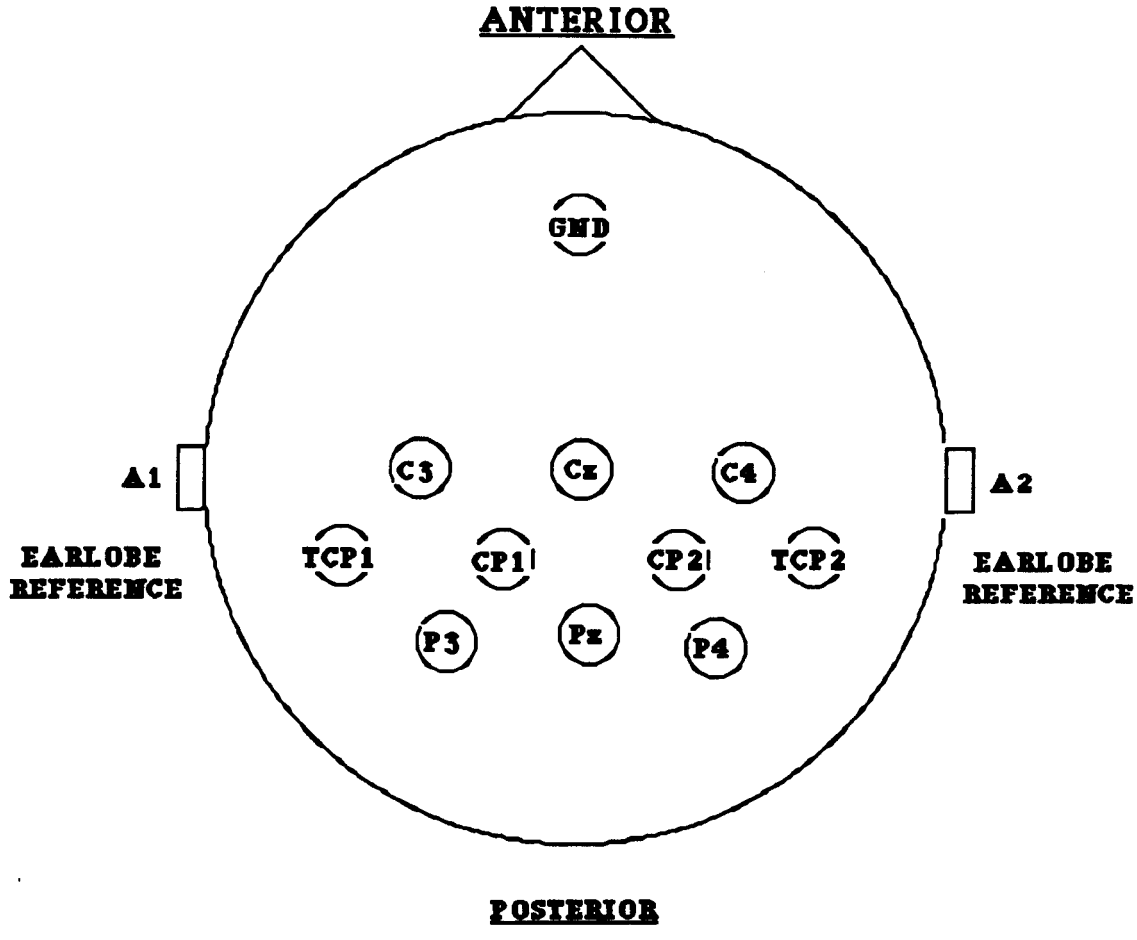


FIGURE 8: SUBSET OF ELECTRODES ANALYSED IN EXPERIMENTAL STUDY 2



4.9.10. These 10 electrodes were chosen because they derived from what little evidence was available and also because they were a slightly larger set than that described by Van Toller *et al* (1990). The reason for choosing a larger set was to allow for the wide individual variability in infant skull size and headcap fit. These were important factors, because it meant that the correspondence between skull landmarks and electrode positioning was much less certain in infants than adults. This meant that these variables had somehow to be balanced and it was felt that this subset of 10 of the 28 possible electrodes was a reasonable compromise. In this way, those electrodes subject to the most variability due to anatomical factors would not be used. Only those electrodes on the skull vertex were located similarly in most subjects.

Data Analysis

4.10.1. As described previously, one of the main aims of the data handling techniques had been to find a parametric analysis technique that was both practical and statistically rigorous. As described in the Technical Appendix (Appendix 2), a number of alternatives existed and several of these were audited. However, for the various reasons discussed in this Appendix, these were eventually rejected. The main problem with EEG data is that it contains a vast amount of information; amplitude, phase and frequency to name only three. As discussed in the Technical Appendix, choices over what is the best statistical technique have necessarily to be made. This choice inevitably involves a degree of compromise. Following considerable deliberation over advantages and disadvantages, two methods of analysis were seen as possible candidates. These had been developed over a considerable period, as described in the Technical Appendix (Appendix 2). Both were relatively practical using the mainframe computer. The first of these, **Analysis Method 1**, used variance calculations for all electrodes in all subjects for each odour condition. The second, inferential method (**Analysis Method 2**) involved multiple repeated-measures Analyses of Variance (ANOVAs), with Tukey's test for non-additivity. This test was chosen after balancing the often mutually-exclusive analysis requirements of EEG data. This is discussed in Section 4.11., below.

4.10.2. The first, variance-based **Analysis Method 1** is described at this point. This was a largely descriptive technique, with the aim of showing graphically the degree of synchronisation of the EEG data. As described before, EEG synchronisation had been seen using the topographic maps. It should be made clear, however that 'synchronisation' is not used here in the classic, EEG sense. It refers instead to the observation that subjects tended to respond in a similar fashion to the odour stimuli at about the same time. The aim of this method was to give some preliminary validation to this apparent synchronisation, though without statistical corroboration. The idea was that if there was any between-subjects synchronisation of activity following odour presentation, then the variance of some electrodes would tend to fall at this point. This would then give an early indication of whether a subsequent inferential analysis would show any significance.

Data Analysis Method 1

4.10.3. The steps in this method were as follows. A number of frames were chosen for closer analysis, as mentioned above. These were the frames prior to odour presentation and those during presentation. This formed the analysis 'window', which was definable by the program user (see Technical Appendix). The variance of each electrode for all the subjects in any one odour condition was calculated and ranked, lowest to highest, by the program. Hence, 28 variances were possible; one for each electrode. It was felt that the most accurate representation of activity would be found in the median variances, because of the low susceptibility of the median to outlier values. Hence the choice of those electrodes showing median variances was felt to be the best compromise in trying to summarise such highly variable data. The variance values were converted to standard deviations, which form the ordinate of the graphs. The reason for this was to allow smaller numbers to be used. A small, but representative sample of the electrodes in Odour 1 is shown below (Figure 9-Figure 12)

FIGURE 9

ODOUR 1, MEDIAN STANDARD DEVIATIONS BY FRAMES
ELECTRODE 1 (CZ)

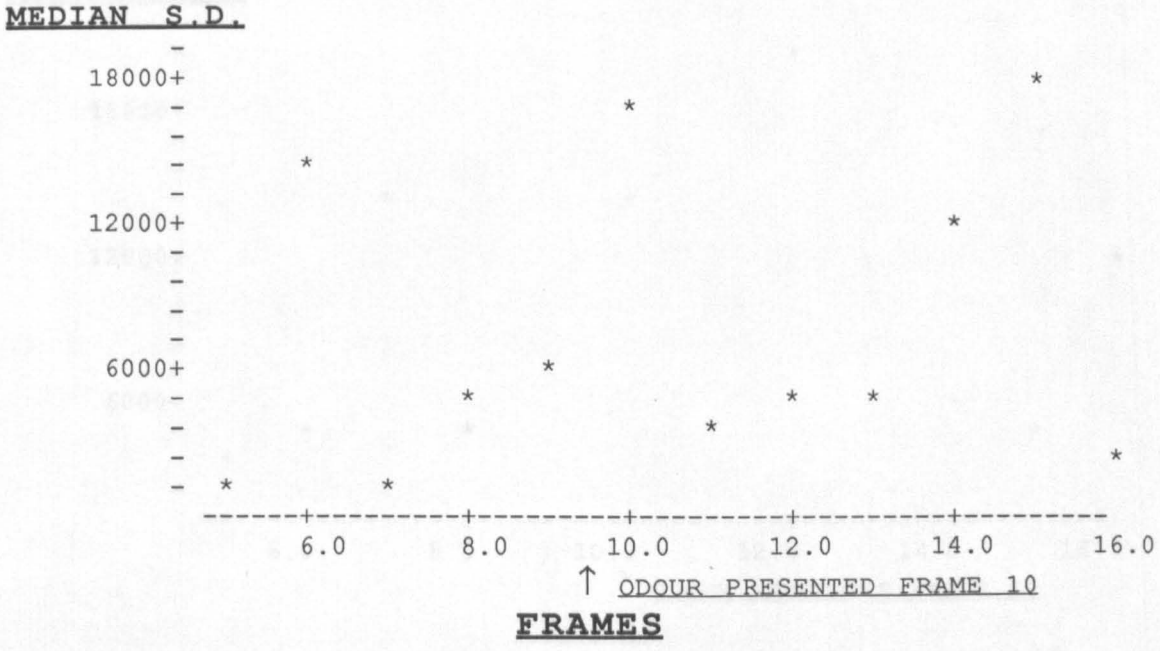


FIGURE 10

ODOUR 1, MEDIAN STANDARD DEVIATIONS BY FRAMES
ELECTRODE 2 (PZ)

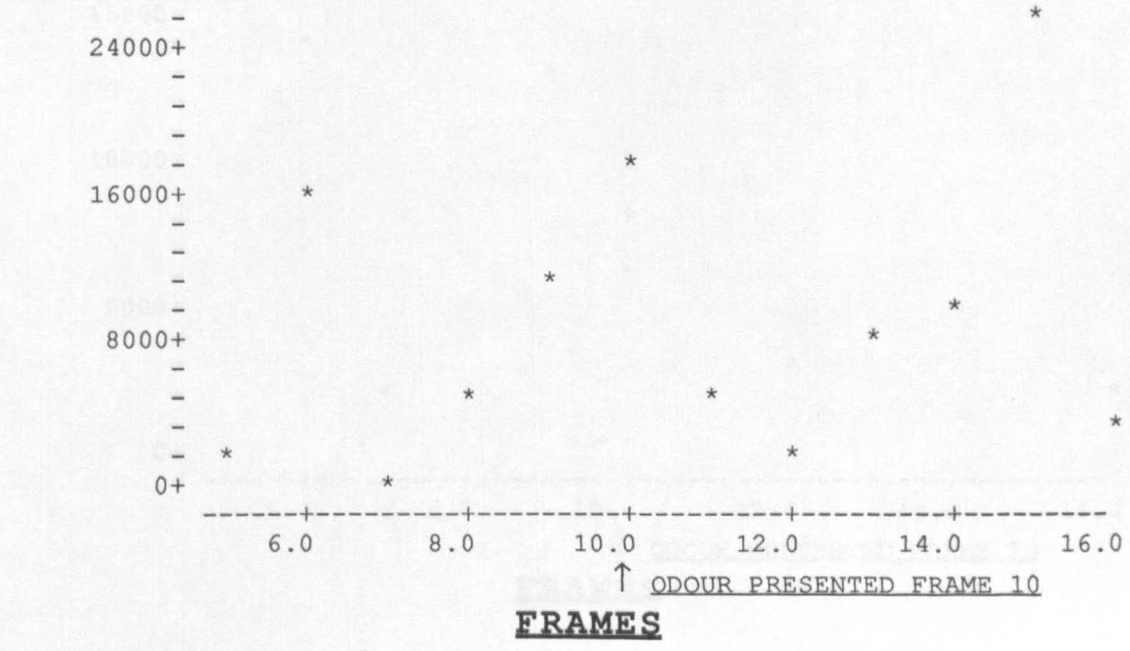


FIGURE 11

ODOUR 1, MEDIAN STANDARD DEVIATIONS BY FRAMES
ELECTRODE 3 (C3)

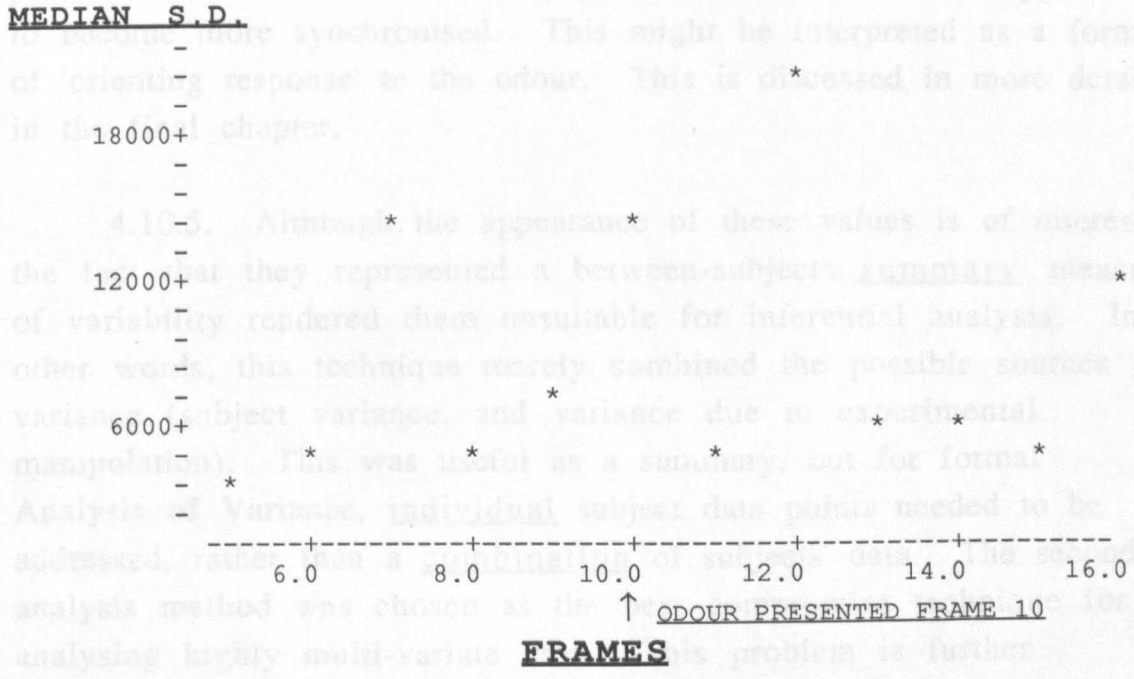
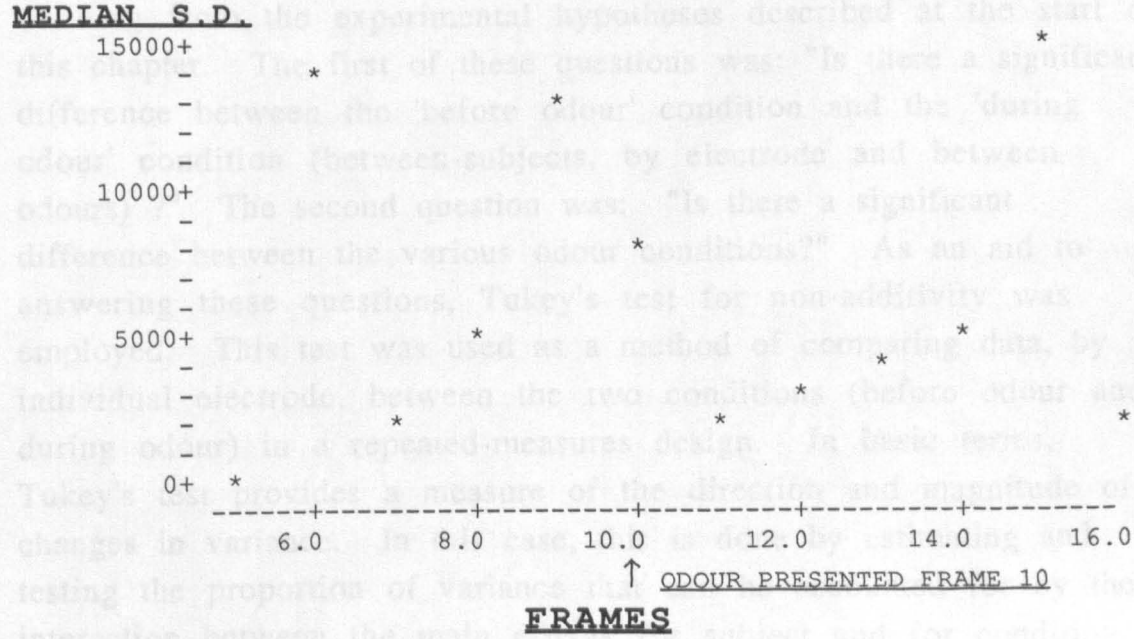


FIGURE 12

ODOUR 1, MEDIAN STANDARD DEVIATIONS BY FRAMES
ELECTRODE 4 (P3)



4.10.4. As can be seen from these graphs, the data variability apparently tended to lessen following in the frames following odour presentation at Frame 10. In other words, the EEG traces appeared to become more synchronised. This might be interpreted as a form of 'orienting response' to the odour. This is discussed in more detail in the final chapter.

4.10.5. Although the appearance of these values is of interest, the fact that they represented a between-subjects summary measure of variability rendered them unsuitable for inferential analysis. In other words, this technique merely combined the possible sources of variance (subject variance, and variance due to experimental manipulation). This was useful as a summary, but for formal Analysis of Variance, individual subject data points needed to be addressed, rather than a combination of subjects' data. The second analysis method was chosen as the best compromise technique for analysing highly multi-variate data. This problem is further discussed in the Technical Appendix (Appendix 2).

Data Analysis Method 2

4.11.1. The aim of this analysis was to answer two questions deriving from the experimental hypotheses described at the start of this chapter. The first of these questions was: "Is there a significant difference between the 'before odour' condition and the 'during odour' condition (between-subjects, by electrode and between odours) ?" The second question was: "Is there a significant difference between the various odour conditions?" As an aid to answering these questions, Tukey's test for non-additivity was employed. This test was used as a method of comparing data, by individual electrode, between the two conditions (before odour and during odour) in a repeated-measures design. In basic terms, Tukey's test provides a measure of the direction and magnitude of changes in variance. In this case, this is done by estimating and testing the proportion of variance that can be accounted for by the interaction between the main effects for subject and for condition.

4.11.2. If it is hypothesised that subjects' EEG will respond to an odour stimulus, then it is likely that some will react in one direction (increasing or decreasing in amplitude) and some in another. This is due to unavoidable subject variability. This could be described as the 'subject response vector'. The magnitude of the response, especially the case in EEG, would also differ between subjects. This is the response magnitude. Tukey's test can be interpreted as providing a measure of both response vector and magnitude It was hence used in Analysis Method 2. In order to do this, further data manipulation was needed. As explained above, only the Delta waveband was used, and a subset of the electrodes. Data were edited by hand, with rigorous criteria for artifact. Any anomalous or suspect data were re-coded as 'missing data' in a form acceptable to the MINITAB statistical analysis program used. These artifact criteria were the same as described above. Even with the relatively small sample available for analysis, the data set for all subjects in both 'before odour' and 'during odour' conditions for each odour produced 56 data files. These each contained either 252, or 308 actual data points; the former in the 'before odour' condition, the latter in the 'during odour' condition. The total data set for all subjects was thus 15 680 values. Addressing each odour condition in a subjects-by-conditions ANOVA, for <u>each frame</u> would have produced an unacceptably large number of separate analyses.

4.11.3. Hence it was decided to choose what would be the most representative frames in each condition. The frames chosen were the final frame before odour presentation (frame 9) and the frame synchronous with odour presentation (frame 10). This reduced the size of the analysis problem considerably, but still required a separate ANOVA for each electrode, to see if any provided significant differences. Hence, ten between-subjects repeated-measures Analyses of Variance were performed for <u>each</u> odour condition; odours one to five. Each of these ANOVAs was duplicated using Tukey's test for non-additivity.

4.11.4. Odours six and seven were not analysed because the data sets were too small. As described above, odour seven was used a control stimulus in one subject. The graphical output from this

data set bore no resemblance to graphs produced when the subject received odorous stimuli. This was interpreted as meaning that the operation of the odour delivery system by itself produced no cortical response.

4.11.5. Before the results are reported, a point needs to be emphasised. This concerns the size of the sample. As explained above, not only were there rigorous criteria for subjects' participation in the study, but also the data that these subjects contributed was further screened before entering the data set. These factors, in combination with time constraints, ensured a relatively small sample of data survived to be analysed. However, the corollary of this is that these data were of the best quality obtainable under the circumstances.

Results of Study 2

4.12.1. Results of the Analyses of Variance described above are as follows. To simplify the results of multiple analyses, they are given in tabular form. Table 4 gives the F-ratios for the straightforward repeated-measures ANOVAs. Table 5 gives the F-ratios for the same analyses, but using the Tukey's test. Table 6 gives the mean amplitude values, with standard deviations, for all odour conditions, by electrode.

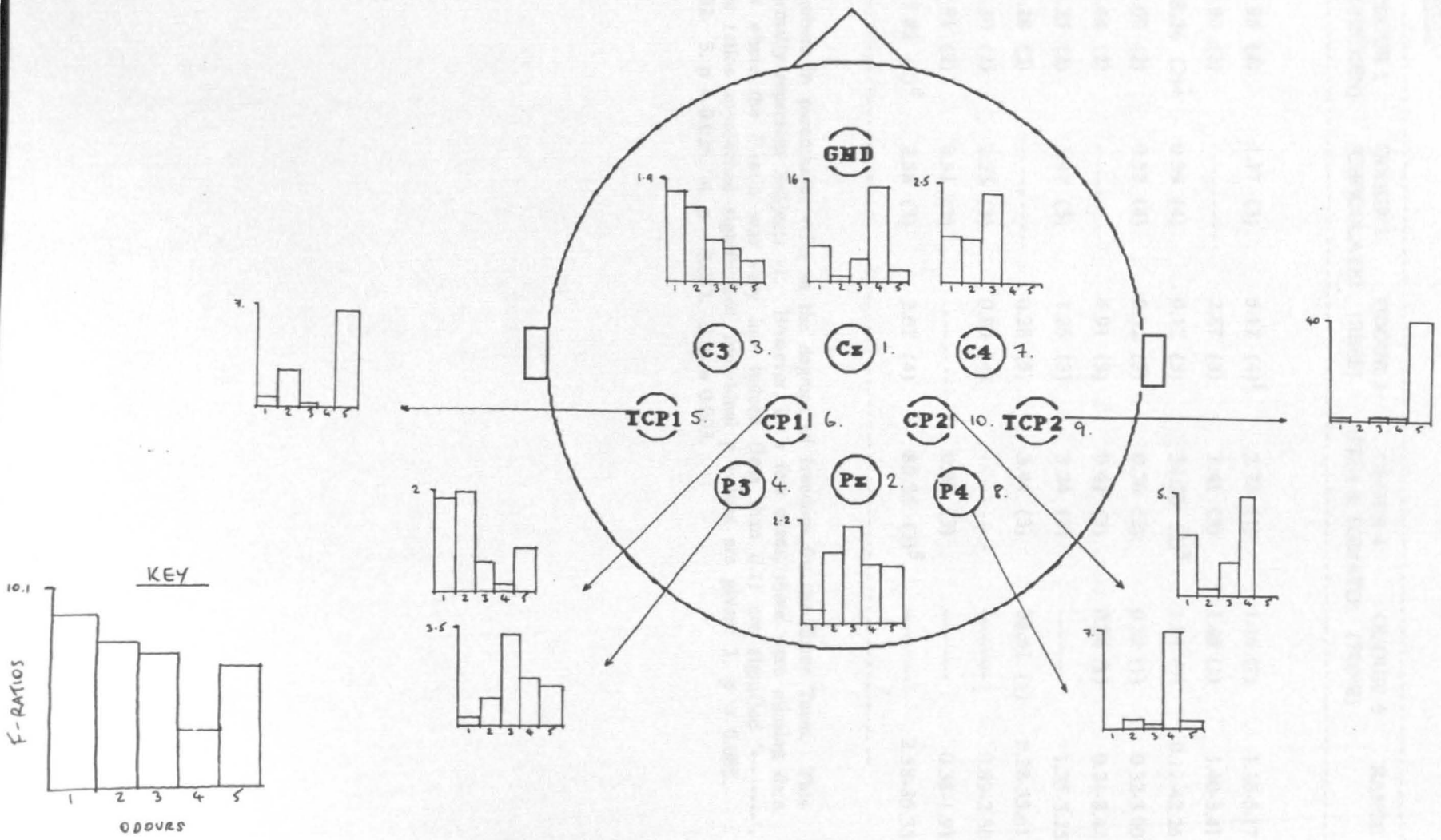
TABLE 4: F-RATIOS (AND ERROR TERM DEGREES OF FREEDOM) EXPERIMENTAL STUDY 2 (BEAM). REPEATED-MEASURES ANOVA COMPARISONS OF FRAME 9 (BEFORE ODOUR) AND FRAME 10 (ODOUR PRESENTATION).

ELECTRODES

	ODOUR 1 (CHICKEN)	ODOUR 2 (CHOCOLATE)	ODOUR 3 (BEEF)	ODOUR 4 (FISH & TOMATO)	'ODOUR' 5 (TONE)	RANGE
1	5.32 (3)	0.79 (4)	2.58 (5)	15.43 (2)[1]	2.59 (2)	0.79-15.43
2	0.70 (3)	1.44 (4)	2.15 (4)	1.16 (4)	1.17 (2)	0.70-2.15
3	1.86 (3)	1.68 (5)	0.87 (6)	0.56 (3)	0.33 (2)	0.33-1.86
4	0.72 (3)	0.97 (5)	3.48 (6)	1.88 (4)	1.83 (2)	0.72-3.48
5	0.98 (3)	2.88 (4)	0.20 (4)	---------	6.72 (2)	0.20-6.72
6	1.86 (3)	1.93 (4)	0.51 (4)	0.12 (4)	0.62 (1)	0.12-1.93
7	1.15 (3)	1.10 (4)	2.37 (6)	---------	---------	1.10-2.37
8	---------	1.32 (4)	0.87 (5)	6.78 (4)[2]	1.11 (1)	0.87-6.78
9	0.21 (3)	---------	1.50 (5)	1.19 (4)	38.15 (2)	0.21-38.15
10	2.30 (3)	0.12 (4)	1.68 (5)	4.68 (4)[3]	---------	0.12-4.68

N.B. Numbers in parentheses refer to the degrees of freedom for the Error Term. This would normally represent Subjects -1. However, in a few cases, there were missing data. Occasions where the F-ratio was very low indeed (less than 0.1) are signified '--------'. Where the ratios approached significance, individual p values are given: 1. $p = 0.059$. 2. $p = 0.060$. 3. $p = 0.097$.

F-RATIOS. GRAPHICAL SUMMARY OF TABLE 4: EXPERIMENTAL STUDY 2 (BEAM). REPEATED-MEASURES ANOVA COMPARISONS OF FRAME 9 (BEFORE ODOUR) AND FRAME 10 (ODOUR PRESENTATION).



F-RATIOS, GRAPHICAL SUMMARY OF TABLE 5: EXPERIMENTAL STUDY 2 (BEAM). REPEATED-MEASURES ANOVA (WITH TUKEY'S TEST FOR NON-ADDITIVITY). COMPARISONS OF FRAME 9 (BEFORE ODOUR) AND FRAME 10 (ODOUR PRESENTATION).

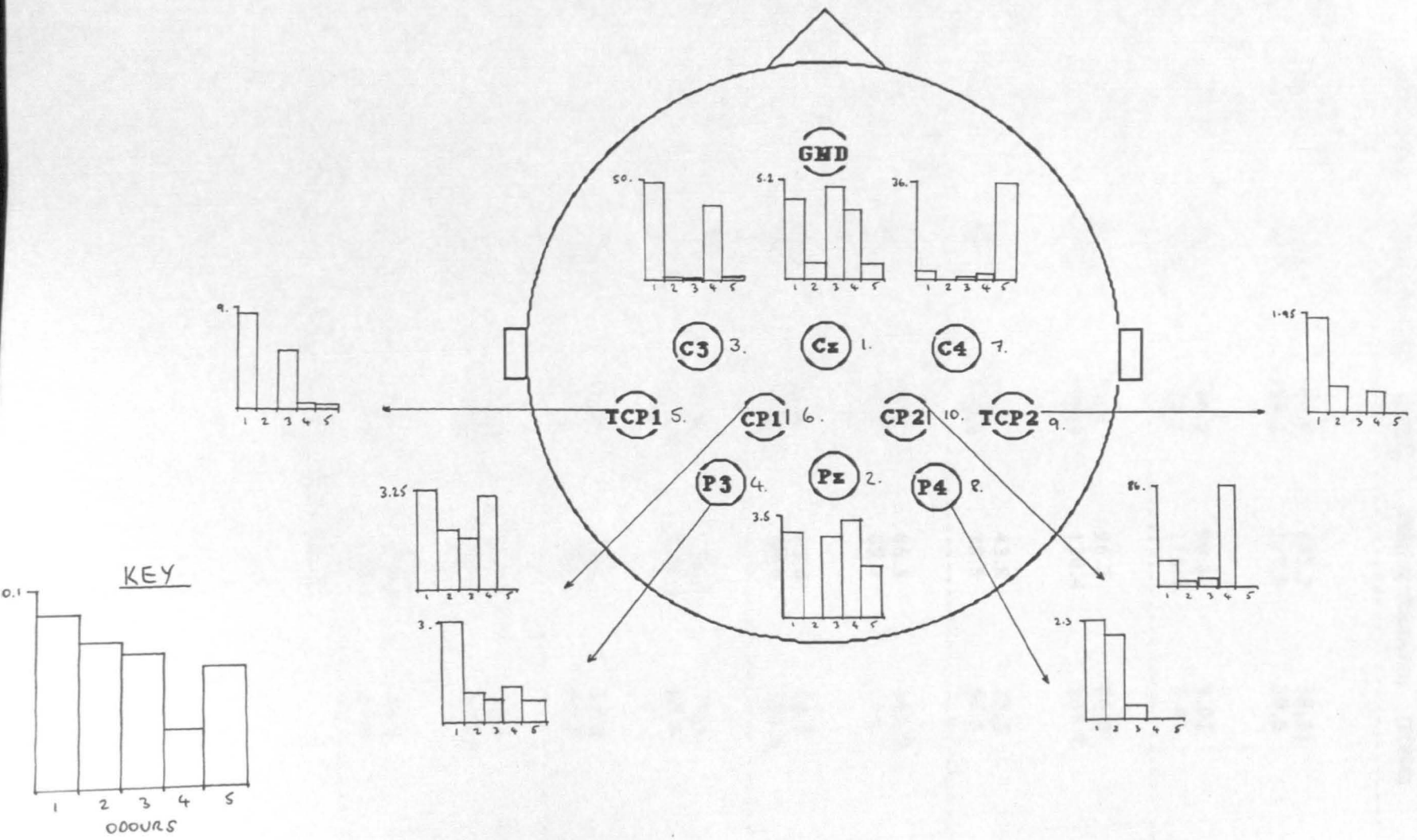


TABLE 6: MEAN AND STANDARD DEVIATION DATA VALUES FOR ELECTRODE SUBSET, EXPERIMENTAL STUDY 2 (BEAM). COMPARISONS OF FRAME 9 (BEFORE ODOUR) AND FRAME 10 (ODOUR PRESENTATION).

	ODOUR 1 (CHICKEN)	ODOUR 2 (CHOCOLATE)	ODOUR 3 (BEEF)	ODOUR 4 (FISH & TOMATO)	'ODOUR' 5 (TONE)
ELECTRODE 1 MEANS					
FRAME 9	149.3	77.8	107.7	137.2	38.33
FRAME 10	163.3	95.6	133.0	117.0	30.0
ELECTRODE 1 S.D.					
FRAME 9	128.8	58.1	140.0	90.8	9.02
FRAME 10	138.7	74.4	111.1	118.1	3.61
ELECTRODE 2 MEANS					
FRAME 9	120.3	78.3	99.2	96.2	86.3
FRAME 10	154.8	91.0	199.7	120.4	133.7
ELECTRODE 2 S.D.					
FRAME 9	44.8	42.2	69.5	43.8	27.0
FRAME 10	103.2	26.2	172.5	78.8	52.5
ELECTRODE 3 MEANS					
FRAME 9	76.0	49.2	56.7	86.5	162.0
FRAME 10	129.0	89.2	75.7	69.7	116.7
ELECTRODE 3 S.D.					
FRAME 9	60.7	45.8	44.8	83.0	43.7
FRAME 10	136.5	62.3	51.6	39.6	101.4
ELECTRODE 4 MEANS					
FRAME 9	112.7	80.8	79.4	136.0	72.3
FRAME 10	137.5	112.5	110.0	174.6	85.0
ELECTRODE 4 S.D.					
FRAME 9	69.1	92.6	83.2	146.1	50.9
FRAME 10	113.2	67.8	73.0	174.1	60.7
ELECTRODE 5 MEANS					
FRAME 9	135.2	102.0	134.8	167.4	99.7
FRAME 10	165.5	157.4	183.0	167.6	185.0
ELECTRODE 5 S.D.					
FRAME 9	38.6	66.8	159.2	158.5	34.3
FRAME 10	92.1	70.3	178.3	127.7	54.4

ELECTRODE KEY (SEE FIGURE 8): Cz=1, Pz=2, C3=3, P3=4, TCP1=5.

TABLE 6: MEAN AND STANDARD DEVIATION DATA VALUES FOR ELECTRODE SUBSET, EXPERIMENTAL STUDY 2 (BEAM). COMPARISONS OF FRAME 9 (BEFORE ODOUR) AND FRAME 10 (ODOUR PRESENTATION).

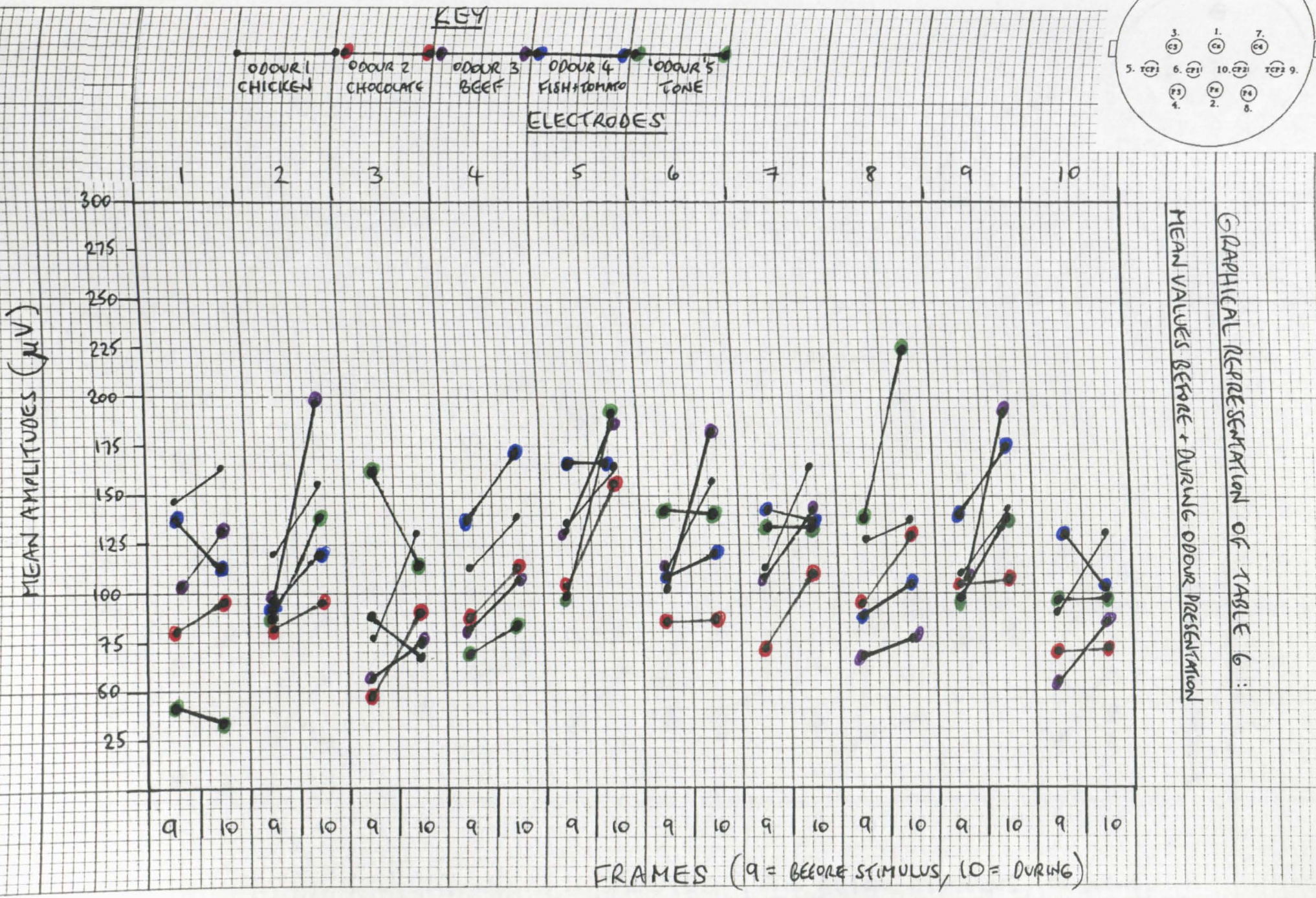
(CONTINUED FROM PREVIOUS PAGE)

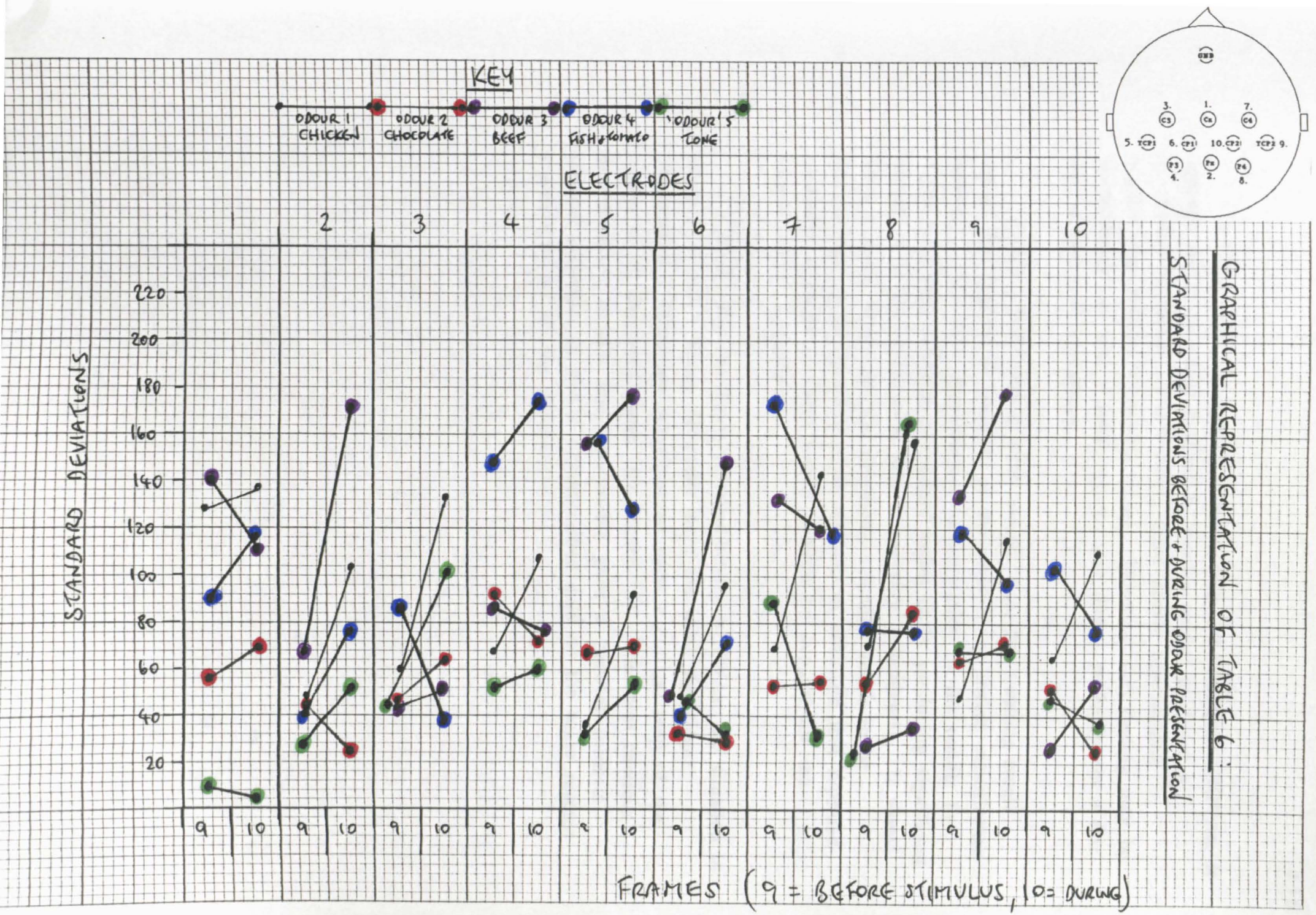
	ODOUR 1 (CHICKEN)	ODOUR 2 (CHOCOLATE)	ODOUR 3 (BEEF)	ODOUR 4 (FISH & TOMATO)	'ODOUR' 5 (TONE)
ELECTRODE 6 MEANS					
FRAME 9	113.7	84.5	102.2	113.8	142.3
FRAME 10	158.5	86.2	179.0	121.0	140.5
ELECTRODE 6 S.D.					
FRAME 9	48.8	36.8	53.8	40.9	47.7
FRAME 10	98.2	31.7	146.4	72.5	37.5
ELECTRODE 7 MEANS					
FRAME 9	114.0	72.8	115.0	145.0	130.0
FRAME 10	166.0	119.8	144.3	142.8	135.3
ELECTRODE 7 S.D.					
FRAME 9	69.9	52.5	131.9	173.2	88.5
FRAME 10	142.8	55.0	120.4	119.8	33.5
ELECTRODE 8 MEANS					
FRAME 9	126.2	95.8	68.8	86.8	134.0
FRAME 10	135.8	128.8	76.0	103.4	225.0
ELECTRODE 8 S.D.					
FRAME 9	73.1	56.6	28.6	78.6	25.7
FRAME 10	158.4	83.8	36.4	77.2	163.0
ELECTRODE 9 MEANS					
FRAME 9	111.2	102.7	105.8	143.0	99.7
FRAME 10	140.8	105.2	189.1	175.4	141.0
ELECTRODE 9 S.D.					
FRAME 9	49.8	62.9	133.5	119.5	66.5
FRAME 10	118.5	72.6	179.2	98.3	66.7
ELECTRODE 10 MEANS					
FRAME 9	87.0	71.5	55.2	127.2	95.0
FRAME 10	128.8	73.8	85.8	103.0	96.7
ELECTRODE 10 S.D.					
FRAME 9	64.5	50.3	25.2	103.3	45.0
FRAME 10	116.4	27.9	53.0	78.7	38.7

ELECTRODE KEY (SEE FIGURE 8): CP1=6, C4=7, P4=8, TCP2=9, CP2=10.

GRAPHICAL REPRESENTATION OF TABLE 6 :

MEAN VALUES BEFORE + DURING ODOUR PRESENTATION



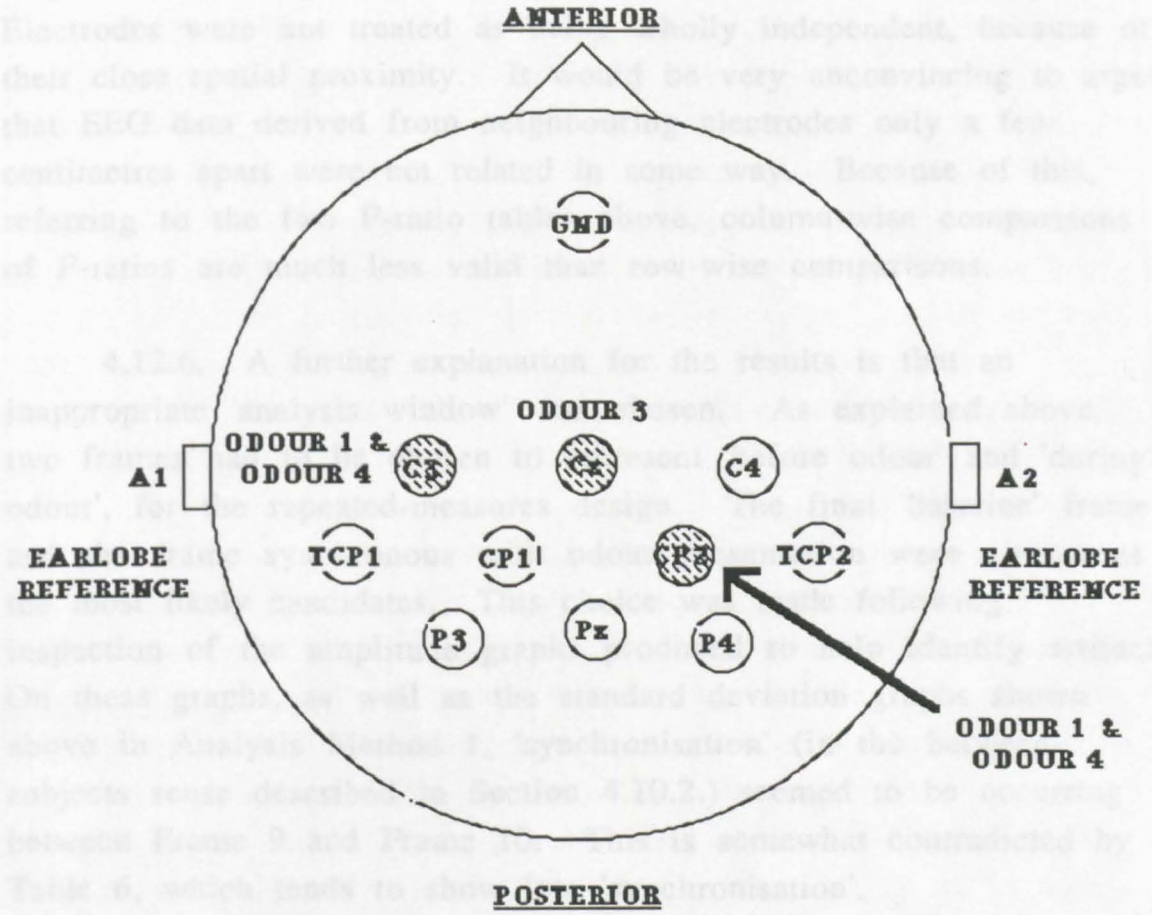

KEY
ODOUR 1 CHICKEN
ODOUR 2 CHOCOLATE
ODOUR 3 BEEF
ODOUR 4 FISH & TOMATO
ODOUR 5 TONE
ELECTRODES
1
2
3
4
5
6
7
8
9
10
STANDARD DEVIATIONS
220
200
180
160
140
120
100
80
60
40
20
9
10
FRAMES (9 = BEFORE STIMULUS, 10 = DURING)
GRAPHICAL REPRESENTATION OF TABLE 6:
STANDARD DEVIATIONS BEFORE & DURING ODOUR PRESENTATION

4.12.2. For the straightforward, repeated-measures ANOVAs, it appears that only odour 4 (Fish & Tomato sauce) produced a consistent response. The F-ratios most nearly approaching significance resulted from this odour. However, this finding may be coincidental and with a small sample size this is probably not a fair test of the null hypothesis.

4.12.3. With such a small sample size, the resulting small values for degrees of freedom of the error terms makes significance unlikely. The most parsimonious interpretation of the results in Table 4 is that, although one odour seems to be producing significant results in several electrodes, this is probably coincidental. It would need a much larger sample, producing a similar result to be convincing. It is therefore more realistic to conclude that repeated-measures analysis of these results showed no support for the experimental hypotheses.

4.12.4. For the Tukey's non-additivity ANOVAs, the situation is slightly clearer. Three electrodes were the most significant 'responders'. These are superimposed upon a cortical map of the subset of 10 described above (see Figure 13). The most cautious interpretation is that these electrodes gave a between-subjects cortical response, in that a circumscribed area on the scalp reacted consistently to all the odours.

FIGURE 13: SUMMARY OF ELECTRODES ACCOUNTING FOR MOST VARIANCE, BY ODOUR



To put it another way, for all the odours, only these three electrodes seemed to be responding when any odour stimulus was presented. As can be seen from Table 6, there appears to be a pattern of response between the electrodes. For Odour 1 (Chicken Dinner), most of the standard deviations (SDs) increased following odour administration. This would seem to indicate desynchronisation, which is addressed in the Discussion section, below.

Discussion

4.12.5. No reliable claims can be made for discrimination between odours on the basis of these results, which is probably due in part to the design of the analyses, as well as the small sample. Electrodes were not treated as being wholly independent, because of their close spatial proximity. It would be very unconvincing to argue that EEG data derived from neighbouring electrodes only a few centimetres apart were not related in some way. Because of this, referring to the two F-ratio tables above, column-wise comparisons of F-ratios are much less valid than row-wise comparisons.

4.12.6. A further explanation for the results is that an inappropriate 'analysis window' was chosen. As explained above, two frames had to be chosen to represent 'before odour' and 'during odour', for the repeated-measures design. The final 'baseline' frame and the frame synchronous with odour presentation were chosen as the most likely candidates. This choice was made following inspection of the amplitude graphs produced to help identify artifact. On these graphs, as well as the standard deviation graphs shown above in Analysis Method 1, 'synchronisation' (in the between-subjects sense described in Section 4.10.2.) seemed to be occurring between Frame 9 and Frame 10. This is somewhat contradicted by Table 6, which tends to show less 'synchronisation'.

4.12.7. However, this might be explained by the fact the Table 6 only covers the two frames nearest to odour presentation, and the graphs from Analysis Method 1 covers a larger 'analysis window'. This lends weight to the possibility that too narrow an 'analysis window' was chosen for Analysis Method 2. For Odour 2 (Chocolate Pudding), the situation is less clear, with some SDs increasing though the majority do not alter or decrease, indicating inter-subject synchronisation. The same tended to be true for Odours 3, 4 and 5.

4.12.8. However, the problem of between-subjects cortical synchronisation or desynchronisation assumes a similar latency period for all odours. If the latency period differed for each odour, then a rigid choice of 'analysis window' might not show this. For

example, it is possible that perceptually more powerful odours, such as the strong cheese stimulus, might produce a more rapid cortical response than others. It is therefore possible that a measure of latency of response should be a dependent measure.

4.12.9. There would, however, be formidable practical problems in measuring this in infants. For example, stimulus presentation would have to be very tightly controlled and measured with a time accuracy of milliseconds. As far as can be seen, this would require state-of-the-art olfactometry and evoked potential measurement. This has been used in adults by Kobal and his colleagues (Kobal & Hummel, 1988). However, as far as is known, such a difficult technique has never been tried with infants. Nevertheless, a possible future method of doing this is discussed in the final chapter.

4.12.10. A further question that arises from these results concerns the definition of artifact. As described earlier, the definitions for this were very stringent. They were based upon what little is known about the normal infant EEG in a non-clinical setting. Indeed, the method for identifying artifact was somewhat intuitive and based on many hours' perusal of the data. Nevertheless, it is possible that what was assumed to represent artifact might in fact have represented part of the response. In other words, a Type II error was unwittingly committed. This view is based on the work of Skarda & Freeman (1987), discussed in the Technical Appendix (Appendix 2). However, this is a very new and uncertain area. Until more information becomes available about the role of Chaos Theory in human EEG, the alternative hypothesis about artifact remains unproven.

4.12.11. However, there is support for the view that the infant cortical activity was not due solely to some arousal phenomenon. This is because Stimulus 5, the tone, showed very low F-ratios indeed (with two exceptions) for all electrodes. This is interpreted as follows. If the cortical activity seen in both BEAM studies had been due to generalised non-specific arousal, then the response to a tone would have been similar to that following an odour. However, this

was not the case. The graphs described in the Technical Appendix (Appendix 2) made this clear. Those for the tonal stimulus looked dissimilar to those from the odour presentations. This was also the case for the topographic maps. The results provide a degree of statistical corroboration. However, it should be reiterated that the very small sample size makes firm conclusions difficult. Nonetheless, those conclusions that can be plausibly made are discussed in the final chapter. In order to obtain a clearer idea of what the results showed, Table 6 is included, to give some idea of the 'response vector'. As discussed above, this could be described as the direction of the variability following odour presentation.

4.12.12. Table 6 aimed to provide a summary of the data values and how they varied after odour presentation. What seems to be happening is that electrodes that are closely related spatially reacted in a similar way to all the odour stimuli. Examples of this are electrodes 1, 3 and 7. This can be interpreted as vindication of the decision not to treat electrodes as levels of an independent variable, as discussed above. In general, all the electrodes reacted more or less similarly, with an increase in data amplitude and increase in standard deviation. Naturally, there were exceptions, with some electrodes showing an opposite pattern. This can probably be accounted for by subject variability. Although these patterns are interesting, few conclusions can be drawn because the small sample size ensured a low level of statistical corroboration. The conclusions drawn from these data are summarised and further discussed in the final chapter of this thesis.

4.12.13. The final study in this series is reported below. As described earlier in this chapter, subjects who were unsuitable for the BEAM studies were usually used in a study concerned with respiration measurement. It had originally been intended to use the respiratory plethysmography (RP) technique as a simultaneous dependent measure in the BEAM studies. However, this did not prove practical as the Imager could not be re-programmed to record RP data. Nonetheless, a number of subjects were tested with the RP technique.

Experimental Study Number Three - Respiratory Plethysmography

Preamble

4.13.1. There were a number of reasons for using Respiratory Plethysmography (RP). Firstly, because it was felt that sufficient experience had been gained with pilot work in the USA to make this a useful study for subjects unsuitable for the BEAM technique. The RP technique has been used by a number of workers in psychology (e.g. Harper, Schectman & Kluge, 1987). This technique has also been successfully employed by a number of researchers in the field of infant olfaction, as discussed at length in Chapter 2. Hence, the scientific basis for this methodology was reasonably well established. The second reason was entirely pragmatic. The RP device was less invasive than, for example, electrocardiographic (ECG) measurement. Furthermore, the cost of the disposable electrodes necessary for any ECG technique to work safely was prohibitive.

4.13.2. Lastly, because the RP was battery powered, its safety was self-evident to the parents of potential subjects. Because of all these reasons, the RP method was seen as a useful technique to test those subjects unsuitable for the Brain Imager. The experimental hypotheses for this RP study were that respiratory pattern, in terms of respiratory frequency or inter-beat interval, would vary between the 'no-odour' condition and the 'odour' condition, and between odour types.

4.13.3. As described earlier, subjects were judged unsuitable for the BEAM study for several reasons related to headcap fit or poor tolerance of the headcap. It was suggested to the mothers that their babies could participate in the RP study instead. It was explained that this was a different, but not inferior technique to BEAM, for measuring infant olfactory responses. Mothers were reassured that not being included in the BEAM study derived from deficiencies in equipment rather than their child. As it happened, all those in this situation agreed to try the second study. It should be noted that RP testing was only permitted when any child that had been crying had

calmed sufficiently to allow it. The mother's explicit agreement was always sought, following an additional briefing on the RP study.

Subjects

4.14.1. A total of 86 subjects was tested, using the same LOR techniques described earlier in this chapter. Mean subject age was 12 weeks from birth, with a standard deviation of 1.2 weeks. As with the other studies, gender and other information about the infants was recorded, but subjects were not subsequently classified on this basis.

Materials and Equipment

4.14.2. Stimulus types were the same as for Study 2. A Densa respiratory plethysmograph (model number IP3-A1) was used in every case. This was a battery-operated device, normally used at home as an apnoea alarm for babies at risk from Sudden Infant Death Syndrome. It was specially adapted for this study, with the assistance of the manufacturer (Densa Limited).

4.14.3. The RP device comprised a sensor and a control box. The sensor consisted of a plastic coated cylinder, 2.5 cm. in diameter and 7 mm deep. One face of the sensor contained a movable portion, which was moved by close contact with the infant's chest wall during respiration, and comprised the signal for the transducer contained inside the sensor. Transduction of the mechanical motion of the device was effected by a magnetic principle.

4.14.4. The sensor was housed in a plastic mounting attached to an elasticated strap, which encircled the infant's chest. In this way, the sensor was kept in close contact with the subject. It did not appear to be either noticeable by the subject, or interfere with respiration in any way. The sensor was connected to the control box by a flexible lead. This control box served to amplify the signal and also to filter out gross muscular movement caused by the subject changing position. The details of how these functions were performed, as well as full details of the sensor are the subject of a patent application and cannot be reported.

Method

4.14.5. The sensor was positioned on the posterior thoracic wall, usually in the midline, level with the axillae. Some subjects needed to be partially undressed by the mother for this. Others had the sensor positioned over an undershirt, though either method produced a clear signal. After a few minutes to allow the subject to become accustomed to the sensor and its strap, testing began when mother and baby were comfortably settled in the Low Odour Room. The mother was briefed as to the function of intercom system, as previously described. As with all other studies, a full explanation of the procedure had been given to the mother and the withdrawal option explicitly provided.

4.14.6. The RP control box was connected to a Grass polygraph (Model 79D) running at 1 cm/sec., by means of a data cable. One channel of this polygraph consisted of the signal from the RP. A second channel indicated time in seconds. The third channel served as an event-marker, triggered by a push-button held by the experimenter. In this way, the time of stimulus presentation could be reliably linked to respiratory response.

Procedure

4.14.7. The mother was told, via the intercom headphones that an odour would become noticeable, but not when this would occur, for the reasons explained in Study 2. She was asked, as before, to avoid any movement and to refrain from any social interaction with the subject. The door to the LOR was then closed. Testing commenced when the subject was judged settled by the experimenter. This judgement was made with the help of the video camera in the LOR and the RP trace on the polygraph.

4.14.8. Each trial was as follows:

Stage 1: Recording commenced. Thirty seconds' of 'no-odour' condition.

Stage 2: Odorous stimulus inserted into airflow synchronous with 31st. second. Event-marker deployed. Stimulus removed after 15 seconds. Event-marker deployed again.

Stage 3: Further 30 seconds' of 'no-odour' condition. Recording terminated.

Inter-trial interval was always at least one minute, and frequently much longer to allow discussion with the mother, changing of subject position or 'winding'. For the tonal stimulus, the paradigm was the same, except that the odour delivery system was not deployed. As with the BEAM study, order of odour stimuli was shuffled between subjects and not all subjects tolerated the situation long enough to receive all stimuli. If a subject became restless or fractious, some brief attempt was asked of the mother to calm the subject. However, this was rarely successful and the usual outcome was that testing was halted. Following the end of the test, debriefing followed the same pattern as for the BEAM study. Some parents asked for a photocopy of the polygraph printout, which was provided.

Data handling

4.15.1. The polygraph output was coded according to the following technique. Two sequential periods of 15 seconds before odour exposure, and the following 15 seconds of odour exposure, were chosen for analysis. These time periods were thought to be sufficiently representative of the total respiration patterns for each subject. The respiration frequency was measured for each time-period of 15 seconds, as were the inter-breath intervals to an accuracy of 0.1 seconds. The reason for measuring these inter-breath intervals was to see whether these would increase or decrease upon odour presentation. If a breath was in progress

during the switch from 'no odour' to 'odour' condition, that interval was ignored and the next breath measured instead. Each data point for every subject in each condition (odour or tone) was thus recorded. Subjects had one or more conditions deleted if excessive movement artifact was noted. In total, 32 subjects had their entire data sets discarded for either this reason, or due to poor recording quality. The sample summary is as follows:

N = 86
n = 54
Males = 27
Females = 27
Mean age (weeks from birth) = 12 (standard deviation 1)

Results

4.15.2. Data were analysed by Analysis of Variance, with repeated measures (before odour and during odour) and Tukey's test for non-additivity. The reasons for using this test were the same as for the BEAM studies. No significant differences were seen between the 'before odour' and 'during odour' conditions for either respiration frequency or inter-breath interval. For this reason, no analysis was attempted for individual odour differences. The means are shown in Tables 7 and 8, below. The data in Table 8 are shown to two decimal places for clarity. As can be seen, the means are very similar.

TABLE 7: SUBJECTS' MEAN RESPIRATION FREQUENCY IN BREATHS PER 15 SECONDS

		MEAN RESPIRATION FREQUENCY IN THE 15 SECS	
ODOUR	NUMBER OF SUBJECTS	BEFORE ODOUR	DURING ODOUR
1	33	9.8	9.6
2	33	10.6	10.3
3	33	10.3	10.1
4	28	11.0	10.1
5	10	9.9	10.3
6	5	9.2	9.4

TABLE 8: SUBJECTS' MEAN INTER-BREATH INTERVALS IN SECONDS

ODOUR	NUMBER OF SUBJECTS	MEAN INTER-BREATH INTERVAL IN THE 15 SECS. BEFORE ODOUR	DURING ODOUR
1	33	1.34	1.31
2	33	1.30	1.31
3	33	1.27	1.20
4	28	1.20	1.28
5	10	1.46	1.32
6	5	1.59	1.42

N.B. For odour code numbers, see Section 4.8.8. above.

Discussion

4.15.3. The most likely explanation for these findings is that the inter-beat intervals (analogous to frequency) were relatively long for the time-frame of measurement. To put it another way, 15 seconds of measurement before and during odour administration permitted only seven or eight measurements in each condition. Even with a respiration frequency of 1 Hz, the maximum possible number of measurements would have been 15 in each condition; one inter-beat interval/second. Most subjects averaged a respiration frequency of 0.3 - 0.5 Hz. It is likely that any experimental effect due to an orienting response, occurring just after the odour presentation, became submerged in the mean of all the inter-beat intervals during this period. Indeed, a number of subjects exhibited one or two anomalously long inter-beat intervals following the odour presentation, though clearly without statistical corroboration.

4.15.4. However, because of the transience of this response (presumably due to an habituation effect), the rapid return of the respiration pattern to the mean resulted in this novelty response being averaged out. Hence the variances before and during odour administration tended to similarity, resulting in no significant differences. A revisited analysis might usefully have compared the

means of the 'before' condition with just the first two or three seconds of the 'during' condition. However, the disparity in the number of data points that contributed to these means would have made any inferential comparisons rather risky.

4.15.5. Another analysis method might have found a difference in these data; for example, amplitude measurement might have detected subtle differences. However, the problem with measuring amplitude of respiration in infants is its vulnerability to artifact. This was found during preliminary RP work on infants in the USA. Any motor movement of the subject would have been erroneously transduced by the RP sensor as respiration. Even simultaneous 'stabilimetry' measurement (see Chapter 2) might not have been sensitive enough to detect, and hence permit elimination of these artifacts. However, if the RP data had been digitised, filter mechanisms would been included in the digitisation process. These could then have been designed to exclude movement not due to respiration. However, at the time when the experiment was carried out, the necessary computer technology was not available in the laboratory.

4.15.6. These results call into question why a similar study, performed in the USA (described in Appendix 1) obtained significant results. However, the experimental design in this study was somewhat different, involving repetitive stimulation with different odorants. Furthermore, the visual component of the stimuli, rather than their olfactory dimension, might have accounted for the results in the USA work. The RP study described above strove to eliminate the visual component of stimulation. However, it is reasonably clear that the RP technique, as described in this experiment is of little value as a dependent measure in infant olfactory testing. It is possible that it might be useful as a non-invasive, corroborative adjunct to BEAM testing in adults. This would especially be the case if the output were digitised, as described above.

4.15.7. This concludes the description of the empirical work for this thesis. The implications of the BEAM work only are discussed in the final chapter.

CHAPTER 5
CONCLUSIONS AND FUTURE DIRECTIONS

Introduction

5.1.1. This chapter draws together the strands of this thesis and outlines the conclusions drawn from the BEAM-based empirical work described in Chapter 4. It aims to critically assess the contribution of this work to knowledge in infant olfaction and suggest improvements and future directions for this kind of research. The chapter is therefore divided into two sections. Section 1 addresses three **Methodological Conclusions.** These are listed below, and discussed in detail later.

1) The BEAM technique has been shown to be a practical method in the psychophysical measurement of cortical responses to odour in the human infant.

2) Human infants at the age of three months show a pattern of cortical activity in response to a small range of food odours.

3) There is evidence that a limited area of the infant brain is responding to these odours.

Section 2 of this chapter addresses **Theory-based Conclusions** that may be drawn from this work and Section 3 discusses **Future Directions.**

Section 1
Methodological conclusions

5.1.2. As discussed in Chapter 2, several techniques have been previously employed to test infant response to odours. They all have advantages and disadvantages, as described. The psychophysical methods of heart rate, respiration rate and 'stabilimetry' measurement all have some relative advantages over the BEAM technique in that they are fairly simple to use and do not require expensive, high-technology apparatus though this is available if

required. Furthermore, these techniques are relatively unobtrusive to the subject. This allows a reasonably naturalistic situation in which to introduce the odour stimuli.

5.1.3. However, despite some apparent advantages, these simpler techniques tend to suffer from problems of interpretation. These are, however, not quite as apparent as those interpretation problems derived from preferential behaviour or facial expression studies. Nevertheless, ambiguous interpretation still affects these earlier psychophysical techniques. It is usually clear that the infant has processed the odour stimuli, as witnessed by the recorded alterations in autonomic parameters. However, the problem remains that these responses are representations of occurrences in the central nervous system (CNS), not the actual, real-time occurrences themselves. This argument of course brings the problem of definition with it. After all, at what level is it currently possible to measure *in vivo* responses in infants? In other words, what constitutes a measured response?

5.1.4. The major advantage of the BEAM technique would seem to be that it does measure cortical events, as far as that is possible. At one level of analysis, BEAM is also recording a representation of CNS events just as much as the older techniques. This is because intervening structures like the skull impede and distort the cortical surface EEG, or electro-corticogram. However, the BEAM method gets closer to the source of the cortical response to odour than other techniques. Despite the technical and practical problems involved with this method in infant testing, BEAM can show the barely-diluted response that the cortex gives to odours.

5.1.5. It was stressed at the beginning of Chapter 4 that the BEAM technique had never before been used to test infant subjects for olfactory responses. Furthermore, it was not known at the start of the experimental work whether it was even possible to use this technique with infants. As described in the Technical Appendix (Appendix 2), much preparatory work needed to be done to turn a largely clinical device into a tool for infant testing. Hence, one of the aims of this research was to evaluate the BEAM technique in its role

in infant testing, in a methodological sense. Therefore, the first conclusion of this thesis is that:

The BEAM technique has been shown to be a practical method in the psychophysical measurement of cortical responses to odour in the human infant.

5.1.6. The justifications for this conclusion are as follows. The first of these concerns parental acceptability of BEAM as an infant testing method. The practical problems of recruitment were essentially the same as for any other infant study. However, what was not known before testing was whether parents would be willing to permit the use of such sophisticated and advanced technology on their infants. For this reason, the actual phrasing of the recruitment letter was crucial and the result of much deliberation. The greatest possible care was taken, when mothers were first shown the Brain Imager, to emphasise the innocuous nature of the technique. The experimenter had to be alert for any hint of 'technophobia' from the mother, and try to allay any fears. The fact that the mothers were permitted to hold their infant at all times probably acted to reassure them.

5.1.7. In this kind of infant testing, it was seen that the experimenter's attitude to mother and baby was crucial. Had there been any pressure to participate, or any doubts about the safety of the technique, then few mothers would have agreed to allow their infant to be tested. In this way, a potentially serious impediment to using the BEAM technique with infants was largely overcome. Of course, parents who initially agreed to participate were *ipso facto* self-selected. Because of this, it was doubly important that every effort was taken to ensure that the testing was a positive experience. In any independent replications of this work, the experimenter's approach to the parents would need to be seen as paramount.

5.1.8. A second practical point that justifies the conclusion in Section 5.1.5. is the use of the electrode headcaps. The main reason for developing these caps was their likely acceptability to the parents. It would not have been justifiable, in a research setting as

opposed to a clinical situation, to use 28 electrodes that had to be glued to the infants' scalp. This technique might have resulted in better electrode impedances, though, as described in Chapter 4 and the Technical Appendix, this is doubtful. However, the parents would certainly have not found such electrodes acceptable and very few infants would have been tested. Nevertheless, as described in Chapter 4, headcap fitting needed to be of a very high standard to ensure good results. It was clearly this need for scrupulous selectivity in subject testing, in order to ensure good headcap fit, that resulted in a small sample size. This problem is further discussed below.

5.1.9. Infants could therefore be recruited and tested satisfactorily, but empirical experience had shown the need for reducing extraneous variables as far as possible. As described in Chapter 4, if the BEAM technique were to used in babies, then some way had to be found to reduce visual and other extraneous stimulation. In adults, this can easily been done with the use of blindfolding and auditory masking with 'white noise' (Van Toller *et al*, 1990). However, it is unlikely that such techniques would have been tolerated by infants. Because of this, it was the use of the Low Odour Room (LOR) in combination with the BEAM technique that allowed odour testing. Replications of this study would clearly need to take account of this.

5.1.10. Despite the difficulties of testing infants with BEAM, it is feasible with the right equipment. The main advantage of this technique over others is that the responses are measured where they occur, as argued above. In the history of infant olfactory testing, this technique is examining the most centrally-located responses. There is no need to try and decipher facial expressions, interpret 'general motor response', or unambiguously attribute 'preferential head-turning'. In this way, BEAM could be said to provide a view of where behaviour in response to odour originates. However, that is not to say the responses seen are much less ambiguous than with other techniques. Little is known for certain about the origin of EEG, as discussed elsewhere.

5.1.11. Until more is known about where EEG data derive from, the use of BEAM must be limited to measuring signals of unknown provenance. Furthermore, because of the low correlation of electrode position to underlying brain structure, little can be said about what part of the brain is producing the signals. This is discussed in more detail below. However, it became clear during the testing programme that a bonus from this infant work was that a catalogue was also being made of the normal infant EEG. As discussed elsewhere, there is little in the EEG literature on the appearance of the normal infant EEG in this age-group. This stems from the mainly clinical orientation of EEG in infancy. Because of the design of the studies described in this thesis, a small database of 'baseline' EEG from unstimulated infants has been collected. This may be added at some stage to the infant EEG literature.

5.2.1. Despite the uncertainties of EEG, the responses seen in this work do appear to derive from odour stimulation. This results in the second conclusion that:

Human infants at the age of three months show a pattern of cortical activity in response to a small range of food odours.

The results from the graphical representation of the data showed a reasonably consistent set of responses to the odours (see Plate 4, Figure 5 and Figures 9-12 in Chapter 4, and Figures 14, 15 in Appendix 2). These does not appear to be due solely to a cortical 'arousal' phenomenon. If this were the case, then the response to a non-olfactory stimulus would be similar to that of the odour stimuli. In fact, the response to a tonal stimulus did not resemble odour responses in any way. This is corroborated by Figure 16 (Appendix 2) and the results described in Chapter 4. However, as discussed below, this does not imply that the responses seen to odours were necessarily unique. This is because the conclusion about 'arousal' is drawn from data provided by a limited number of subjects. Nonetheless, it is important to point out that the need for stimulation in a non-olfactory modality, to preclude the generalised 'arousal' response, was recognised early on. The results from the tonal stimulus do appear to corroborate the hypothesis that was developed

from this earlier work. This hypothesis was that response would be different between odour and tonal stimuli.

5.2.2. Conclusions as to the nature of the cortical response patterns must necessarily be limited. This is due to two factors. The first of these factors is the small number of subjects and the second is the analysis period chosen. As previously explained, individual variations in EEG patterns are large, both within- and between-subjects. Hence it would need a much greater sample size to be able to draw firm conclusions about the reliability of the responses. It was this realisation that precluded the use during the testing programme of 'mini-experiments'. Such multiple, small-scale studies might have been possible with a well-established psychophysical technique and would have allowed modifications to experimental design to be incorporated in the light of findings. However, the BEAM technique was being used for the very first time in infants, so there was no independent research to draw upon for guidance. During the research programme, changes in experimental design could only be made when there were sufficient data upon which to base conclusions. Because of the rigour of the criteria for headcap fit, discussed previously, the number of available subjects was limited in the time available for testing. These factors conspired to prevent more, small-scale studies being carried out. Future research would naturally be able to draw on the experience gained during the work reported in this thesis.

5.2.3. However, as regards the nature or meaning of the cortical responses seen, the small sample size caused problems of interpretation. There are too many variables within a small sample to say much more than that most subjects exhibited some response to the odours. This statement is generally corroborated by the results. Though few of the repeated-measures, Tukey's test comparisons reached significant levels, at least some did, which is gratifying in a small sample. The interpretation placed on this is that there was a rapid cortical reaction to the odours.

5.2.4. Quite how rapid this reaction was could not be assessed directly, because of the scan-time of the Brain Imager. As the

Imager collected data over a period of 2.56 seconds, very short-latency events typical of cortical sensory activity were not well shown. This problem is discussed in Chapter 4. The response seen in the data was probably due to an 'echo' of cortical reactions measurable in milliseconds. With present technology, measurements of such rapid and transient events is not possible without using Evoked Potentials (EP), as discussed in Section A2.1.8. of the Technical Appendix. It would not be practical to use EPs for measuring odour response in infants due to uncertainties about the onset of odour delivery.

5.2.5. The second of the two factors identified as causing problems of interpretation concerns the selection of the analysis interval. The period that was chosen for statistical analysis, as discussed in Chapter 4, might have given an unrepresentative view of the cortical response. Because of the constraints of the repeated-measures analysis design, a somewhat narrow interval was chosen. It is possible that only a part of the cortical response to the odours took place during the two frames selected for analysis, as the latency of any cortical orienting response to odours in infants is not known. However, the evidence from the studies reported in this thesis suggests that the latency is relatively short, and that the main part of the cortical response occurs in the 2.56 seconds following odour presentation.

5.2.6. This response is almost certainly a variety of the 'orienting response' seen in reaction to a novel stimulus by most organisms. There is debate as to whether the orienting response is cortically mediated in infants (Rosenblith & Sims-Knight, 1985, pages 272-274). However, if the responses seen in this study are orienting responses, then presumably their measurement on the cortex lends support to the view that the orienting response is cortically mediated. A possible explanation for the responses seen is that they are due to an 'olfactory orienting response'. This would be a pattern of cortical activity <u>specifically</u> elicited by odours. Because of the small sample size, there is insufficient conclusive evidence for this. If such an entity as an 'olfactory orienting response' exists, such a small-scale study would be unlikely to demonstrate it. However, the

available evidence appears suggestive. After all, the responses to a tonal stimulus looked quite different from those due to an odour stimulus. However, because of the limited evidence, the most parsimonious explanation is that the cortical response seen in this study is a variety of orienting response that might have special features associated with odour perception. Much more work would be needed to demonstrate any unique qualities to the response to odours. This would involve further specific comparisons of responses to odours over responses to auditory, or some other sensory stimulation. Furthermore, much more would need to be known about the cortical EEG appearance of the infant orienting response.

5.3.1. It would seem from the results that a small number of electrodes on the infant scalp responded reliably across all the odours. As discussed in Chapter 4, these are close together on the vertex of the skull, suggesting the following conclusion:

There is evidence that a limited area of the infant brain is responding to these odours.

Lorig & Schwartz (1988) stated that adult EEG activity in response to odour would probably be seen in the frontal regions of the brain. However, as explained elsewhere in this thesis, it is very difficult to comment in infants on the meaning of EEG in relation to underlying brain structures. This is further complicated by the variations in fit of the electrode headcaps. However, it is interesting that the few electrodes that showed significant differences were in similar locations to those found to react in adults (Van Toller *et al*, 1990). This allows speculation on the developmental aspects of odour perception.

5.3.2. The reasoning behind this is as follows. The main anatomical differences between the infant and the adult brain probably relate to size, degree of neuronal interconnection and myelination. This may be particularly related to inter-hemispheric communication (Yakolev & Lecours, 1967; cited in Fox, 1985; de Schonen & Bry, 1987). Nonetheless, the structural similarities are sufficient to allow developmental comparisons to be made. In this

way, the main areas of activity in infant EEG terms may foreshadow those that have been found in the adult (Van Toller *et al*, 1990). This might even occur in an asymmetrical fashion, following evidence from gustation work in infants (Davidson & Fox, 1982; Fox & Davidson, 1986). It could therefore be speculated that broad areas of the infant brain may respond in a similar, perhaps 'immature' fashion to the adult brain, following odour stimuli. It is therefore possible that activity in small areas of the adult brain is presaged or anticipated in the infant brain.

5.3.3. This is naturally somewhat speculative, but the evidence certainly suggests it. It may even be the case that the infant response to odours is more clearly seen than that of adults. The reasons for this would be two-fold. The first concerns brain size and the second concerns 'cognitive contamination'. Because the infant brain is smaller and less complex than the adult, any signals deriving from deep generators, or dipoles would tend to be of a greater amplitude. This is because there would less intervening brain and a less dense skull to cause attenuation of the signals en route from deep to superficial layers. Presumably the 'inverse square law' of signal propagation operates in this way. It is clear from the evidence in this thesis that the amplitude of the EEG waveforms was often very much higher than those seen in adults. Hence, any EEG data deriving from the olfactory region deep in the telencephalon would be less diffuse and therefore a 'purer' representation of olfactory processing. However, it should be noted that very recent evidence from adults localised a 'trigeminal generator' on the surface of the cortex (Hummel & Kobal, 1990).

5.4.1. The second point concerning the relative 'purity' of the infant EEG signal deals with psychological structures. This assumes that the degree of cognitive ability is less in three month old infants than adults. The complexity of the 'cognitive structures' of memory, reasoning and language in the adult might further serve to attenuate the EEG signal deriving from olfactory structures. A clearer, less equivocal response may be seen in infants because they do not have to consider past mnemonic associations with odours, or make hedonic judgements, or try to name odours. All of these essentially cognitive

activities might serve to attenuate the cortical response to odours in adults, but not infants.

5.4.2. These kind of speculations about the nature of the cortical odour response in infants might even be susceptible to empirical evaluation. It might be possible to record adult odour responses, measured by BEAM, whilst asleep. This would presumably remove some of the cognitive component from the adults' responses, provided the REM sleep-stage was avoided. These responses could thus be compared with those of infants under similar conditions. The hypothesis could be therefore be tested in a non-invasive and ethical fashion.

5.4.3. A final question in this section is concerned with the nature of the cortical response concerns anatomical localisation of the cortical response to odour. As discussed elsewhere in this thesis, it is very hard to do this, due to uncertainties over the correlation of cortical structures and surface electrodes. This is despite the work in cytoarchitectonic mapping done in the adult by Brodmann (Kolb & Whishaw, 1990). However, Kolb & Whishaw also state that it is uncertain whether the olfactory system has any representation on the cortex. If this is the case in adults, then the infant situation is also likely to be obscure. This is because of the lamentable lack of infant histological evidence discussed in Chapter 1. Any statements about localisation of olfactory function in the human infant would be only likely to derive from great technological advances.

5.4.4. It is possible to speculate in this area. A system involving very high-resolution EEG combined with high-speed Positron Emission Tomography (PET) or three-dimensional magnetencephalography (MEG) might permit accurate localisation. Such types of combined brain imaging systems are not possible yet, but may soon be so. Advances in high-temperature superconductivity are likely to result in much more powerful brain imaging systems.

5.5.1. To summarise these conclusions in terms of the original experimental hypotheses, some support is lent for the one that stated:

> *"BEAM could be used to show significant differences between a baseline condition and an odour condition across subjects".*

Whether this type of orienting response is qualitatively different from any other kind remains unanswered. It would seem unlikely that there is anything wholly unique about response to odour, despite the 'biological significance' aspect discussed in Chapter 3. The response to food odour shown in this study might simply be generalised from responses to any 'biologically significant' odour. This is discussed below.

5.5.2. Because of the reasons discussed in Chapter 4, there is little inferential statistical support for the other experimental hypothesis:

> *"Cortical response patterns would differ significantly according to the odour type used".*

Because of this, there is no evidence from these results for the view that cortical response in infants can be used to demonstrate odour discrimination as such. However, there is less than complete support for the null hypothesis either. This is because of the small sample size. It is possible that the few subjects who were successfully tested showed an unrepresentatively high degree of EEG variability. It was for this reason, as well as because the sample size virtually equalled the odour number, that significant differences between odours was not shown.

5.5.3. A way to demonstrate this would be to repeat the experiment with only two odours, a different and smaller electrode subset and a large sample of subjects. This strategy would so increase the power of the experiment as to make inter-odour differences more likely. Two distant electrodes could be chosen, perhaps TCP1 and TCP2. These electrodes are therefore less likely to

produced spatially related data and could be treated as independent for the purposes of analysis. Further suggestions for improving infant BEAM methodology are discussed later in this chapter.

Section 2
Theory-based conclusions

5.6.1. The next set of conclusions relates to the theoretical model expounded in Chapter 3. As explained, it was suggested that the reason for early infant olfactory competence, or 'infant mesosmia' could be related to accelerated intra-uterine maturation of the olfactory system. This was argued for in terms of General System Theory and particularly, 'emergent properties'. The gist of the argument was that prolonged interaction between the foetal olfactory system and the chemosensory environment of the amnion resulted in a synergy. This synergy was the 'emergent property' of this interaction and resulted in olfactory competence that was precocious in relation to other sensory modalities.

5.6.2. In terms of the results of the BEAM studies, it is difficult to say for certain whether the model is supported or not in the age-group tested. This is because, by three months of age, infants are becoming visually dominant. This was shown quite clearly by the results of Experimental Study 1 as well as numerous other studies into infant visual capabilities. Because of this, comparisons between sensory modalities would be needed. In order to demonstrate continuing olfactory competence at this age, some general measure of sensory competence would need to be established. In other words, it would have to be established that at any particular age, one sensory system was more advanced than any other. Thus it could be shown that an infant's olfactory ability was demonstrably better than its visual ability for any developmental stage. Quite how this could be done is a difficult question.

5.6.3. This is because of the number of possible variables that would have to measured to allow cross-modality comparisons. For example, acuity, discrimination ability and sensitivity threshold would presumably all have to be measured. This may be possible with the visual system, but there are insufficient data at present to

say that this would be possible with the olfactory system. The studies described in this thesis have made a start on this aspect of infant olfactory psychophysics, but much more work is needed. At this stage, much clearer evidence about the olfactory discriminative abilities of infants would be needed. If one assumes that a good measure of the sophistication of a sensory is its ability to discriminate, then at three months of age, there is far more evidence for the visual system (Slater, Morison, & Rose, 1982; Werner & Lipsitt, 1981; Kaplan, & Werner, 1987; Aposhyan, Kaplan, Peterzell & Werner, 1988; Slater, 1990).

5.6.4. Evidence for the discriminative ability of the olfactory system at three months of age is limited. The corollary of this is that the cross-modality comparisons described above are not yet possible. The work described in this thesis has made a start in this area, but clearly much more work is needed before the case for 'infant mesosmia' in this age-group is proven. At the moment, the term remains a useful way of conceptualising the cross-modality, as well as cross-species comparisons in the developing infant. The methodology described in this thesis has the potential for lending support to other parts of the model described in Chapter 3 of this thesis. However, before this is discussed in the section entitled '**Future Directions**', the contribution of the work in this thesis has to be evaluated.

5.7.1. The evidence from the relatively small-scale studies described in this thesis has advanced knowledge about infant olfaction a little. In the context of previous research, the experimental work is firmly in the psychophysical tradition identified in Table 2, Chapter 2. Hence the main contribution has to been to advance knowledge about activity in the central nervous system in infants exposed to odours. Because this was the first use of BEAM in this area, questions remain about its usefulness that could not be answered in small studies. For example, the responses shown by the infants were not as unambiguous as was first hoped. With hindsight, this is not surprising, given the highly multivariate nature of infant EEG data. A much larger sample, as discussed elsewhere would help to overcome this to some extent.

5.7.2. The BEAM method was originally seen as a way of addressing real-time central nervous system events as they occurred. The technique has largely been vindicated for use in infant olfactory research, though a large-scale replication is needed to validate this. The main drawback of the technique is its expense and the time devoted to retrieving the EEG data is a usable form. Hopefully, both these factors will be lessened by advances in microchip technology.

5.7.3. As with any study, improvements become clear at all stages. The next section will address these and discuss ways of testing areas that are implied by the 'infant mesosmia' model described in Chapter 3.

Section 3
Future directions

5.8.1. Two areas implicit in the theoretical model mentioned above are discussed in terms of informed speculation. The first of these concerns the concept of biological significance and the second deals with the possibility of intra-uterine olfactory learning.

5.8.2. As described in Chapter 2, much experimental effort has been devoted to the study of the role of maternal odour. The notion of 'biological significance' of odours has been advanced in the animal models described elsewhere in this thesis. If there is an analogue in the human, then the odour of the mother is a good candidate, for the reasons discussed in Chapter 3. Although the experiments described in Chapter 4 did not show conclusive evidence for discrimination of such biologically significant odours as food, the BEAM technique clearly has the potential to do so. All that would probably be needed, as discussed before, would be a smaller range of odours combined with a much larger sample of infants. If significantly different BEAM data were shown between these odours, that could be interpreted as discrimination. An habituation/dishabituation paradigm, as used by many infant workers, could realistically be used.

5.8.3. A subsequent experiment could use maternal odour compared with odours derived from 'alien' lactating mothers in the same way. The odour stimuli could be collected as described by researchers such as Schaal and Porter. These odours could then be tested on young infants, up to four weeks old for example, using the BEAM paradigm described in Experimental Study 2. The experimental hypothesis would revolve around the ability to discriminate these odours, with differential BEAM response as the dependent measure. This sort of study could be seen as removing some of the ambiguities inherent in previously described work using preferential behaviours.

5.8.4. If such a study were to lend additional support for infant discrimination of maternal odour, then this might generate implications for nursing and medical practices. For example, it might be suggested that premature infants isolated in incubators should be regularly exposed to maternal odour. This might help 'prematures' to thrive, as well as enhance infant-mother 'bonding'.

5.8.5. A further possible implication for this kind of work might be in the area of food preference. This area was not addressed in this thesis, because of lack of evidence from the BEAM studies. However, a longitudinal study might be envisaged. If infants were regularly tested with food odours from an early post-natal stage, BEAM response might be correlated with the development of preference for the odour or taste of a particular food following weaning. This would probably involve combining gustatory and olfactory stimulation. Practical difficulties might make this problematical, but the BEAM technique has recently been considered for use in adult gustatory work.

5.8.6. If food preference could be demonstrated at an early developmental stage, this would have implications for infant diet. For example, if such research demonstrated highly consistent infant preference for a group of odours, then they could be added to baby food. Furthermore, such odours could assist 'difficult' feeders, perhaps those babies who have undergone periods of starvation in the Third World.

5.9.1. Further directions that this research might take are concerned with the technical aspects of BEAM. Such is the speed of advance in computer technology that the three year old system used in the studies for this thesis is relatively dated, though still an advanced system in comparison with many. As described in Section 5.2.3. above, the latency period of olfactory EEG response might be very short. What would therefore be needed to measure this is a BEAM system with a very fast scan-time. If sufficient EEG data could be recorded in less than 2.56 seconds, then very high temporal and spatial resolution would be achieved. The sampling rate, as described in the Technical Appendix, would have to be very high indeed. However, the combination of a very fast (perhaps optical) processor, vastly increased Random Access Memory and improved disk storage would enable this. In this way, uncertainties about the onset of the cortical response to odour could be reduced.

5.9.2. Allied with this idea is the possibility of advanced, solid-state olfactometry. The present technology, as described elsewhere, would not be suitable for infant testing using Olfactory Evoked Potentials (OEPs). This is due mainly to the size and complexity of the equipment and the method of odour delivery (Kobal & Hummel, 1988, 1989; Hummel & Kobal, 1990). However, with advances in nano-technology and genetic engineering, it is possible to speculate about a ultramicro-miniaturised olfactometer.

5.9.3. This device would be about 1 mm. in size and could therefore be secured safely in the anterior nares of infants. Advances in recombinant genetic technology might allow 'tailored' bacteria to release specific odour molecules when electrically stimulated; a sort of 'odour transistor'. This could allow several such 'odorgens' to be located on a silicon chip a few hundred microns across. Hence, multiple odour presentations would be possible with one device. Such a device would sense the time of peak respiratory inspiration, perhaps using artificial cilia as anemometers.

5.9.4. The 'odorgens' would release a specific pure odorant repetitively into the inspired airflow in synchrony with the respiration pattern. This would obviate the need for warming and humidification of odorants, and trained respiration patterns as with current olfactometers. Another part of the device could remotely synchronise an external BEAM device to record OEPs in a similar way to the current method described by Kobal and his colleagues. This device, which would probably be called an 'odour nanotransducer', would be eminently suitable for infant testing because of its unobtrusive size.

5.9.5. To conclude this thesis, it has been shown that a relatively new psychophysical technique, hitherto untried in infant testing, can be used successfully in this area. It is, of course, only a small, first step in an area that promises many years of research. It is hoped that further work will lend support to both the technique and to the model proposed to explain a fascinating area of human development.

HUMAN INFANT OLFACTION:- RESPONSES TO FOOD ODOURS MEASURED BY BRAIN ELECTRICAL ACTIVITY MAPPING (B.E.A.M)

IN TWO VOLUMES.

VOLUME TWO

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SUBMITTED FOR THE DEGREE OF PhD

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September 1990

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VOLUME TWO

APPENDIX 1

PRELIMINARY INFANT STUDIES CARRIED OUT AT THE UNIVERSITY OF COLORADO

Preamble

A1.1.1. This Appendix gives details of early preliminary work in the field of human infant olfaction, carried out by the author. These studies took place at the University of Colorado, Boulder, USA between 1985 and 1986. All work was done in the Infant Perception Laboratory of the Department of Psychology. The research was carried out with the permission, funding and encouragement of Dr. J. Werner and Dr. P. Kaplan: co-directors of the Infant Perception Laboratory.

Introduction

A1.1.2. The aim of this study was to replicate the facial expression work of Steiner (1979). This work is discussed in detail in Chapter 2 of this thesis. It was hoped to be able to verify that Steiner's technique of decoding facial expressions in response to odour could be used as a reliable response measure in young infants. A preliminary study and two experiments were carried out. The aim of this preliminary study was primarily to test out the equipment and the technique and decide which forms of facial response might be expected. Following preliminary work, it was decided to concentrate upon a narrow range of facial expressions (see Table 9), and use a fairly rigid and standardised experimental method. It was hoped that a consistent and reliable facial repertoire would result if the experimental stimuli were presented in a fixed paradigm, with fixed intervals. It was also hoped that the decoding of the responses on videotape would be feasible. The aim of this would be to see whether any response increment, or even response decrement would obtain across trials. At this stage, it was not known what would really occur.

TABLE 9

RESPONSE CODES AND OPERATIONAL DEFINITIONS

CODE	DEFINITION
B - Head turn	any head movement in any plane.
I - Wide open mouth	gaping, with or without tongue projection.
O - Lips pursed	puckering action - mouth open or shut.
P - Sucking	clear sucking action; may or may not involve hands, fingers or clothing.
X - Drooling	clear excessive salivation. May involve peri-oral region.
Z - Eyes converge/focus	visual fixation upon stimulus.
ALPHA - Masticatory response	chewing motion of jaws.
GAMMA - Sniffing/tachypnoea	may not be obvious and need close attention to respiratory movements.
THETA - Reach and grab	may be un-coordinated.

N.B. Codes are not mutually exclusive.

HEDONIC/AVERSIVE SCORING SYSTEM

Score 5 = hedonic. This may be smiling, prolonged fixation on the stimulus or increased motor activity. Judgement derives from an overall impression of the subject's affective response to the presentation of the stimulus.

Score 3 = neutral. Little or no affective component to facial response.

Score 1 = aversive. This may include frowning, crying or any movement interpreted as the subject's attempt to distance himself from the stimulus. Judgement derives from an overall impression of the subject's affective response to the presentation of the stimulus.

A1.1.3. The second experiment used all the experience gained during the previous studies. It was decided to use a wholly objective measure of response, and to this end a strain-gauge plethysmograph was employed. It was thus hoped that such a measure of largely autonomic response would minimise the subjective component that had appeared in the first experiment.

Method and Materials (Preliminary Study)

Subjects

A1.2.1. A sample of 37 subjects was used, 18 males and 19 females. All were healthy babies, with a mean age of 3.6 months. The range was 2-4 months, plus or minus one week. The subjects were recruited from a large subject pool, created through following up newspaper birth announcements for another study. This study examined visual perception, and a number of subjects in the pilot olfaction experiment were tested within ten minutes of participation in the visual study (n=24). Each visual experiment lasted between four and five minutes.

A1.2.2. The subjects' parent or guardian was present throughout the whole of the experiment, and written consent had been given. All parents were given the explicit option, as part of the briefing, of withdrawing at any time without incurring rancour. All subjects were screened by means of a questionnaire for prematurity of birth, perinatal illness or surgery, known allergies, general health (including the presence of upper respiratory tract infection at the time of testing), and finally their state of weaning. No subjects were excluded from the experiment on the basis of the questionnaire, though four were described as being premature. Additionally, seven had experienced perinatal difficulties, including *icterus neonatorum* (perinatal jaundice) and foetal distress syndrome. No subjects had known allergies, and only four had minor colds or coughs. Nine of the subjects had already started solid food, usually rice cereal. The rest were breast-fed, or bottle-fed on baby formula. Four subjects had the test terminated before completion because they became too upset to continue. Two sets of dizygotic twins were included in the sample.

Experiment 1: Subjects

A1.2.3. For this experiment, 13 subjects were used. Recruitment was from the same source as described above. This sample included six males and seven females, all within one week of three months of age. On the basis of the preliminary questionnaire, five subjects had perinatal problems, two had allergies to lactose, and one had a minor cough at the time of testing. Two sample members had started rice cereal. The rest were breast, or bottle-fed. Additionally, seven subjects had participated in the visual study immediately prior to the olfactory experiment. In two cases the experiment was halted before completion as the subject became upset.

Experiment 2: Subjects

A1.2.4. This experiment used 16 subjects, thirteen males and three females. All were within one week of three months of age, and included one set of dizygotic twins. Only one subject had an allergy (lactose), though three had perinatal problems similar to those mentioned above. Only four subjects had started rice cereal. Two subjects were tested after having participated in the visual study. In one case the test was aborted due to subject distress. Lastly, a small control group was tested (n=3), all were females. This group received twenty-four presentations of the control stimulus, whilst connected to the plethysmograph. Experimental method was identical with that of Experiment 2.

Apparatus

A1.2.5. All subjects were tested in the same laboratory. This was a window-less, but ventilated room, equipped with variable-intensity incandescent lighting. It was heated to approximately 20 degrees Celsius. The subjects were seated in an infant seat mounted on a powered dental chair which could be raised and tilted as desired. The infant seat was equipped with a safety strap to limit the subject's range of movement relative to the stimuli, and prevent accidental falls. Once seated, the subjects faced a relatively featureless partition wall, painted matte black. The sole objects in their forward field of vision were a small perspex screen, measuring 20 cm by 20 cm, and the lens of a low-light video camera ; the body

of which was concealed by the wall. The video camera was used to film the subjects' facial expressions.

A1.2.6. Mounted to the left side of the subjects, and slightly above their head, was a digital display count-up timer, visible to the video camera (though not to the subjects), and remotely activated by the experimenter simultaneously with the presentation of the first stimulus. The facial expressions of the subjects were monitored by the video camera, (Concord Communications Systems, model MTC-21), which was a low-light model to obviate the need for movie lights. The face of the subject was displayed on three monochrome video monitors, and recorded on standard VHS-format videotape by means of an unmodified JVC video cassette recorder.

A1.2.7. Subjects in Experiment 2 were also fitted with a chest-band electrode connected to a plethysmograph (Parks Electronics, model 270-A). This device was in turn connected to a standard strip chart recorder (Houston Instruments), running at 50 cm per minute. It was fitted with a remotely activated event marker to indicate stimulus presentation. In this way, any changes in respiratory rate or pattern in response to the stimuli could be recorded and later analysed. Stimuli consisted of two commercially-available food flavours (almond and anise) which were colourless, and alcohol-free. They were used in their undiluted form, having been chosen from a small range for their relative strength and acceptability when tested informally on adult colleagues. Polyethylene glycol; an almost odourless, colourless and harmless chemical (see Odour Glossary, Table 3, Chapter 2) used in the food manufacturing industry, acted as the control stimulus. All stimuli were presented on 15 cm long, cotton-tipped swabs, held in the hand of the experimenter. A disposable, odourless plastic glove was worn on this hand to conceal any trace odours originating from the skin of the experimenter. All subjects were tested under similar room lighting levels, in a quiet environment.

Procedure

A1.2.8. Subjects were prepared for the test, if necessary, by feeding and diaper-changing. It did not prove possible to control the time of day at which the subjects were tested. This was left to the mother, who suggested the time at which the child would be optimally alert and hopefully co-operative. Once judged relaxed, subjects were placed in the experimental chair and strapped loosely in. Where used, the plethysmograph chest-band was fitted, and the device calibrated. It should be noted that the electrode did not hamper the child in any way, and appeared to be absolutely undetectable in use.

A1.2.9. A one or two-minute tape was made of the subject under conditions of no experimental stimulation, to act as a baseline for later rating of facial expressions. A baseline recording of respiration pattern was also made at this time. Once this was completed, the timer was turned on and stimulus presentation began. This was accomplished by the experimenter standing to the right, and slightly behind the subject. The tip of the cotton swab was held within about 2.5 cm of the anterior nares, in the midline and at the level of the upper lip. At no time was this swab allowed to touch the subject.

A1.2.10. Stimuli were presented according to the following paradigm : five presentations of the control stimulus, three seconds each, with up to three seconds between presentations. Immediately following the final presentation of the control stimulus, 10 presentations of the first experimental stimulus were made (the 'habituating' stimuli). The same time-intervals as above were employed. Two presentations of the second experimental ('novel') stimulus were then made. These followed on immediately from the previous stage, again employing identical time-intervals. After this, two repeat presentations of the first experimental stimulus were made, exactly as before (the 'dishabituating' stimuli). Lastly, five further presentations of the control stimulus concluded the series. Overall, the subject received a total of 24 stimulus presentations, over a period of about 120 seconds. The video cassette recorder was

turned off, plethysmograph disconnected and the subject removed from the experimental chair. The presentation paradigm is summarised below in Table 10:

TABLE 10:
STIMULUS PRESENTATION SUMMARY
USA PILOT WORK (1). EXPERIMENT 2

Presentation number (presentations 3 secs. each, 3 secs. apart)	Event
1)	control stimulus (P.E.G.)
2)	control stimulus (P.E.G.)
3)	control stimulus (P.E.G.)
4)	control stimulus (P.E.G.)
5)	control stimulus (P.E.G.)
6)	experimental stimulus 1
7)	experimental stimulus 1
8)	experimental stimulus 1
9)	experimental stimulus 1
10)	experimental stimulus 1
11)	experimental stimulus 1
12)	experimental stimulus 1
13)	experimental stimulus 1
14)	experimental stimulus 1
15)	experimental stimulus 1
16)	experimental stimulus 2
17)	experimental stimulus 2
18)	experimental stimulus 1
19)	experimental stimulus 1
20)	control stimulus (P.E.G.)
21)	control stimulus (P.E.G.)
22)	control stimulus (P.E.G.)
23)	control stimulus (P.E.G.)
24)	control stimulus (P.E.G.)

N.B. 'P.E.G' = polyethylene glycol (q.v.). Designation of 'stimulus 1' = almond, 'stimulus 2' = anise. Average total time for 24 presentations = 120 secs.

Response coding

Preliminary Study

A1.2.11. All the videotapes from this study were studied by an independent rater, on a single-blind basis. The principal experimenter second-rated all the tapes, to assist in judgements over which categories were valid with this sample. The rater was familiar with the repertoire of infant facial expressions, but blind to stimulus type. Rating was accomplished by replaying the videotape for each subject as often as necessary, and making a frequency count of all

the facial expressions defined by Ganchrow, Steiner & Daher (1983). This was done for the total number of stimulus presentations (24), not for discrete presentations. Rating of ambiguous expressions was facilitated by a freeze-frame facility on the videotape recorder. Event-timing was possible by use of the digital count-up timer mentioned above. It quickly became clear that a number of the expression-categories were redundant, or very infrequent. Those categories with a low frequency (less than two per subject) or low inter-rater correlation coefficient (Pearson $r < 0.25$) were dropped. This left a repertoire of nine rateable expressions derived from experience in the pilot study (see Table 9). Additionally, as may be seen from Table 9, a rating of the affective component of each presentation was made.

Experiment 1

A1.2.12. With the aid of the newly operationalised expression repertoire, each presentation of each stimulus for each subject was rated. Frequency of each response was counted, by two independent raters. One of these was naive to the study, and was told simply to rate according to the response repertoire. However, both raters operated on a single-blind basis. Their frequency-counts were then correlated.

Experiment 2

A1.2.13. For this experiment, all subjects were tested by means of the strain-gauge plethysmograph. Videotapes of their facial reactions were made simultaneously, as in Experiment 1, but they were not rated. The reason for this was that it had become too time-consuming to do so in the limited period available. Not only that, but preliminary analysis of the facial expression data had revealed some very low inter-rater correlations and wide, within-subject variation. Interpretation of the plethysmograph recordings was as follows. By means of the built-in event marker on the strip-chart recorder, each stimulus presentation interval was delineated. The mean interval was 3.45 seconds. Responses in each interval therefore represented a number of respiration cycles in a fixed unit of time. Within each interval, positive (that is, above baseline) excursions were counted according to two methods. Method A

counted only those excursions with discrete positive and negative components, ignoring very small, 'step-like' deflections. Method B counted all excursions, however small, and regardless of their direction above baseline. Neither method counted negative excursions falling below the baseline. Although Method B yielded higher figures than Method A, the trend was the same, and the **Pearson r** correlations between the two counting methods ranged from 0.51 to 0.87. Additionally, each printout was second-rated to check reliability amongst observers. The range of Pearson r inter-rater correlations was 0.80 to 0.97. It was decided to only employ Method A as a dependent measure, because of the subjective component in judging what constituted a very small excursion. Amplitude was not used as a dependent measure, because the very great sensitivity of the plethysmograph rendered it vulnerable to recording artifact caused by the subject moving limbs or altering position. This tended to lead to very large anomalous excursions, which distorted the data.

Results

Experiment 1

A1.2.14. Inter-rater correlations (Pearson r) were calculated for each stimulus category. This was done on a within-subjects and between-subjects basis. Results showed wide inter-subject variation, and low inter-rater correlation. Values for the latter, in terms of each discrete category between subjects ranged from -0.42 (Category Gamma, which was 'sniffing') to 0.43 (Category Z, which was focusing on the stimulus): $p < 0.05$. Inter-rater correlation was highest for hedonic score. Pearson r for this was 0.64 ($p < 0.05$). In other words, none of the response categories proved to be reliable either between raters, or even between subjects. Indeed, there were a number of negative correlations between raters. Even for the highest inter-rater correlation, this was less than 0.50. The relatively high inter-rater agreement for hedonic/aversive expression merely seems to show that adult observers can tell when babies are reacting pleasurably or otherwise to a stimulus.

Experiment 2

A1.2.15. Results for this experiment demonstrated significant effects when the data were subjected to a between-subjects ANOVA. In every case, 'response' meant a change in respiration pattern (breath cycles per unit time). These effects were in response to novelty following the introduction of the last of five control stimuli. This was designated an 'orienting' response. Values derived from the ANOVA were $F = 4.67$, d.f. = 1, 14, $p < 0.05$. The other significant response was that to the novel stimulus following the ten 'habituating' trials. In other words, this response took place on presentations 16 and 17. Values were $F = 4.85$, d.f. = 2, 28, $p < 0.05$. There was a trend towards habituation over trials 6 to 15, but this was not statistically significant. Interestingly, two distinct groups emerged. These were the 'accelerator' group and the 'decelerator' group. The first group tended to speed up their respiration over the ten 'habituating' trials. The latter group tended to slow down over trials. However, since the figures obtained for these two groups were not significant, as mentioned above, it is not known whether this was an artifact of some kind. Dishabituation was not demonstrated with these subjects. In other words, response change on trials 18 and 19 was not statistically significant.

Discussion

A1.3.1. The results of the first experiment appeared to demonstrate that the use of facial expressions as a reliable dependent measure was not appropriate in this age-group. However, this may not be a widely generalisable conclusion in that the results of this experiment may have derived from a number of extraneous factors. Steiner (1979) seemed to show that there was a rather limited, indeed almost stereotyped repertoire of expressions that could be elicited in the age-group he used. It could be argued that as his subjects were neonatal, and hence at an immature stage of ontogeny, then a limited repertoire would be expected. This is not, of course, the exact point made by Steiner. He argued more for a built-in, 'hardwired' system of facial expressions as an aid to infant-maternal interaction and communication. This system is located, so he asserted, at a fairly low level (cognitively speaking) of the brain.

It would seem reasonable to speculate that the pre-existing expression repertoire would tend to be modified and expanded as neurological development progresses rapidly in the first few months of life. This might account for the findings of this experiment in terms of the wide subject variability in response to a fixed series of odour presentations. This would tend to derive from different subjects of similar ages undergoing neurological maturation at varying rates. Then there is the whole question of prior experience with odours, which could not have been reasonably controlled for. It is quite possible that facial expressions, seen as responses to stimuli, are learned and hence superimposed on the repertoire that the child seems to be born with.

A1.3.2. Another explanation for the results obtained in Experiment 1 might have been due to poor, or ambiguous operational definition. Despite considerable discussion over the results of the pilot study, decisions as to which response categories to keep and which to abandon were somewhat subjective. In fact, those which were the most vague tended to be dropped, in the hope that reasonable inter-rater reliability would ensue. The aim was to leave only those categories which could be consistently agreed upon. However, the results showed that this was something of a vain hope. The probable explanation for this was that even with fairly rigidly-defined descriptions of what constituted each category, subjective interpretation by the raters was unavoidable. This may have accounted for the number of negative inter-rater correlations.

A1.3.3. Another problem was that the total number of responses tended to be fairly small. This did not permit sufficient practice for the raters to become familiar with the repertoire, and hence caused disagreements in judgement between raters. It is possible that another study, using more subjects might overcome this difficulty. A further problem was a technical one, and derived from equipment limitations. The camera that was used was an old model, and produced a very poorly-defined image. When this image was videotaped, the definition suffered even more. This led to interpretation problems and exacerbated the difficulties of the raters. Lastly, rating of facial expressions was exceedingly time-

consuming and labour-intensive. The demands upon the concentration of the raters was considerable, and led to errors of interpretation. It was therefore preferable to use a more objective dependent measure.

A1.3.4. With this in mind, Experiment 2 was performed, using strain-gauge plethysmography, which was felt to be just such an objective measure. As stated above, this demonstrated significant effects. The orienting response was anticipated, and was in itself a response to novelty. This was because of a noted habituation to the control stimuli, manifested facially by an attention decrement over the five presentations. This habituation was presumed to be visually mediated, as the stimulus was odourless as far as could be determined without sophisticated analysis. In terms of the definition of Engen (1982), the response decrement to the control stimuli occurred because they were not significant to the subject. The novel stimulus (presentation number six) caused a response increment presumably because the subjects could discriminate that it differed in only one dimension, namely that it had an odour. This is what is termed dishabituation according to the view of Sokolov (1963). Though the trend towards habituation over trials 6 to 15 was not significant, it was nonetheless interesting.

A1.3.5. However, a plausible alternative hypothesis to explain these findings was that of the visual component of the stimuli. The well-known visual dominance of infants at the testing age might have caused the 'novelty' response shown. Although the experimenter took care to remain behind the subjects, and to ensure that the odour stimuli were visually uninteresting, it is likely that the changes in respiration were visually mediated. This casts doubt on any contention about these results providing evidence for some kind of 'olfactory orienting response'.

A1.3.6. To summarise the work described above; this was a valuable introduction to the problems of infant olfactory testing. The main conclusion to be be made is that the facial expression paradigm described above is as yet unproven. In other words, this did not constitute a wholly successful replication of Steiner's work.

TECHNICAL APPENDIX (APPENDIX 2)

Introduction

A2.1.1. This Appendix is divided into two main sections. The first covers aspects of EEG acquisition and brain imaging. The second covers data handling and software development. This is further covered in Chapter 4. of the main thesis. This first section deals with the problems inherent in traditional EEG measurement and how these would make its use in psychophysiology very difficult. The second section discusses the very considerable development needed to turn the Brain Imager from its design role as a clinical tool, into a workable method for testing infant olfactory responses.

Section 1: EEG acquisition and Brain Imaging

The EEG signal in Brain Imaging

A2.1.2. The problem of data analysis in psychophysiology is not confined merely to the quantitative. There is also a problem of the data quality. As with many psychophysiological data, the content comprises much that derives from extraneous sources; in other words, 'noise' or artifact. Because the human cortex produces so much in the way of electrophysiological output, a high proportion may not apparently relate to the critical experimental measure of interest (though see discussion below). Its identification is crucial to avoid accidentally including artifact in analysis, leading to the kind of 'garbage in = garbage out' problem discussed by Maus & Endresen (1979). Possible sources of 'noise' include:

1) cortical activity related to cognitive, perceptual and vegetative functions.

2) extra-cortical electrical potentials such as electromyographic (EMG), electrocardiographic (ECG) and electro-oculographic (EOG) activity. These are generally millivolt voltages, as opposed to the microvolt output of EEG.

3) ambient electrical interference deriving from electrical and electronic equipment, either local or remote.

4) impedance between recording electrodes and EEG sources.

This is a function of distance from cortical surface to electrode, extra-cellular fluid and intervening tissue (notably bone) resistance, skin resistance, electrolyte composition and electrode construction. There is also a considerable amount of resistance from the wires connecting headcap to Imager, as well as the internal resistance of integrated circuitry. However this was effectively the same for all subjects and so may be discounted. The impedance of individual subjects of course differed considerably. The net result of all these sources was a generally unfavourable signal-to-'noise' ratio. The Imager 'recordist' is faced with identifying and extracting a very small signal of unknown characteristics in a 'chaotic', 'noisy' environment.

A2.1.3. However, the problem of definition still has to be addressed. This defines what constitutes 'signal' and what constitutes 'noise' in the human cortex. The traditional view is that 'noise' is random, distorting the signal and therefore needs to be eliminated in order to permit meaningful analysis. This view is challenged in a paper by Skarda & Freeman (1987). These authors, in a complex and difficult work, first define the context:

> *"The elemental phenomenon that must be dealt with in olfaction, as in all brain physiology, is the background activity manifested in the "spontaneous" EEG... and unit activity of neurons throughout the CNS. How does it arise,and what role does it play? This activity is exceedingly robust..."* (page 164).

It should be noted that these authors are not discussing EEG as recorded on the human scalp, but rather on the surface of the rabbit olfactory bulb and olfactory cortex. Nonetheless, the thesis of Skarda & Freeman may well be applicable to humans. They argue that background 'noise' in the brain is not something that is random, but rather is 'chaotic'. It is also a necessary condition for sensory

analysis and learning in this modality. These workers define the situation thus:

> *"Chaos is indistinguishable from random noise in appearance and in statistical properties, but it is deterministic and not stochastic"* (page 165).

A2.1.4. The essence of their position is that the so-called 'noise' found in the bulbar EEG signal is fundamental to perception, rather than an intrusive phenomenon that has to be filtered out before research can discern its meaning. How far this situation is true in the scalp EEG of human infants is an unknown quantity. If, as Skarda & Freeman claim, chaotic behaviour is a pre-requisite to olfactory learning, then a period of intense learning (such as in the human infant) would be accompanied by much cortical chaos. Findings reported in this thesis certainly agree with this. However, it is difficult to decide what proportion of chaotic behaviour (in terms of the EEG signal) is due to extraneous sources, as listed above. The parsimonious explanation for the poor 'signal-to-noise' ratio found during brain imaging is that it derives from extraneous sources such as impedance and poor electrode contact. There is, after all, no empirical evidence as yet for Skarda & Freeman's theories in the human being. Nonetheless, evidence may yet be found, and this would revolutionise work in EEG. The current situation is still concerned with identifying a small needle in a large haystack.

A2.1.5. The problem of extracting the signal of interest from an electrically 'noisy' or 'chaotic' background is dealt with in fields like vision research by repetitive stimulation, and averaging of the resultant signal. This has the effect of causing the signal due to the stimulus to 'stand out' from the background 'noise'. The technique is called evoked potential (EP). This is not yet suitable in infant olfaction research, for the following reasons, which embraces a discussion of the pros and cons of two techniques of addressing olfaction research in general.

A2.1.6. If a psychologist is using any form of EEG to investigate psychophysiological events, then one is faced with a choice. This

choice derives from the nature of EEG itself and is concerned with the nature and origin of the EEG signal. There is considerable debate as to what generates the observed scalp EEG signal (Offenloch, 1975). The received wisdom seems to be that no one is completely sure about this. This view is shared by Morihisa (1985), who states that there is no agreement on what generates surface EEG. This view is corroborated by Walter (in Dolce & Künkel, 1975, page 67), who makes the point that EEG must always be linked to observed behaviour to be meaningfully interpreted. Furthermore, Walter states that any results derived from EEG analysis derive largely from the experimenter's choice of models. Several methods for solving this problem have been suggested, including numerous complex mathematical solutions which are discussed in Dolce & Künkel (1975) and Pool, Aronofsky, Finitzo & Barr (1989).

A2.1.7. However, the difficulty remains that, although surface EEG derives from somewhere in the brain, there is no agreement as to where this is. Hence, the psychophysiologist is faced with this problem when attempting to interpret findings. One can either enter the debate amongst electrophysiologists as to the nature of EEG, or accept that the question is still open. The latter position seems the more sensible for the psychological researcher, who is usually not an electrophysiologist. One is thereby treating the brain (at least in EEG terms) as a 'black box', but this may be unavoidable until the exact nature of the human EEG is fully understood.

A2.1.8. A further choice derives from the signal-to-noise ratio described above. Two approaches to this problem can be identified. The first approach involves repetitive stimulation with odorants to produce an averaged EP, as used by Finzenkeller (1966) and later by Allison & Goff (1967). This involves very tight control of stimulus characteristics (temperature, humidity, saturation etc) and precise presentation using olfactometry.[1] The technique has been brought to its technical zenith by Kobal and his colleagues (Kobal & Hummel, 1988, 1989; Hummel & Kobal, 1990). This method allows reliable Olfactory Evoked Potentials (OEP) and Chemosensory Evoked

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[1] For a review of the history of olfactometers and olfactometry, see Wenzel (1948).

Potentials (CSEP) to be recorded, and absolute thresholds to be determined. However, this requires that a constant airflow be inserted into the subject's nostril, as well as a specified respiration regime and a most elaborate experimental set-up. In other words, a situation remote from everyday experience. Furthermore, such a method would not be suitable for babies, not least because of the ethical and technical problems associated with ensuring a constant airflow directly into the nose and repetitive odour stimulation.

A2.1.9. An alternative approach is less concerned with precise control of odorants and absolute values. Recording of real-time EEG rather than EP's allows more flexibility, and dispensing with elaborate olfactometry permits greater ecological validity. The testing situation more closely reflects the real-life olfactory situation. However, the disadvantage lies in the difficulty, described above, of extracting the response. First of all, what constitutes the response had to be defined. This is problematical enough in adults, but becomes more so with infants because of the lack of a reference point. This means that there is little information available to decide what constitutes a 'baseline' EEG. Subsequent comparisons with the EEG produced during odour stimulation were therefore difficult. However, this methodology is more suitable for use with infants as it mimics the everyday, ambient odour situation. Furthermore, this technique relies upon relative differences in EEG amplitude, as opposed to absolute differences. Relative differences are harder to quantify than absolutes in real-time EEG, because the ratio of change is constantly altering. For this reason, the need for statistical corroboration of EEG findings in infant olfactory testing created a number of challenges. Not least of these was the size of the data sets.

Brain Imaging

A2.2.1. The cortical activity of infants has not hitherto been the subject of intensive study in psychology. There is a fairly large clinical literature[2], mainly relating to perinatal asphyxiation and other cerebral insults. However, few studies have concentrated on charting the normal EEG in infants (Parmelee, Wenner, Akiyama, Stern & Flescher, 1967; Anderson, Torres, & Faoro, 1985). As described in earlier chapters, most of the dependent variables measured in psychophysical studies have been autonomic parameters such as heart rate and respiration rate. These have commonly been used with odorous stimuli of various kinds (see Chapter 2), but have measured peripheral events. Whilst it is clearly true that a change in, say, heart rate reflects changes in the brain's activity in response to a stimulus, it does not measure it directly. Despite current debate about the distributed nature of perception, most psychologists would agree that it occurs somewhere in the brain! However, measuring real-time, rapidly-occurring changes in the brain is notoriously difficult. Many routes have been tried, including radionuclide uptake (Ingvar, 1985, *inter alia*), the largely clinical techniques of Positron Emission Tomography (PET) and Computerised Axial Tomography (CAT),[3] Nuclear Magnetic Resonance (NMR) imaging (Steiner, 1987) and latterly, magnetencephalography (MEG) described by Knuutila, Ahlfors, Ahonen, Hallstrom, Kajola, Lounasmaa, Vilkman and Tesche (1987).

A2.2.2. The problem inherent in most of the above techniques is the very long time-base that has to be employed in order to achieve sufficient resolution. Generally speaking, the minimum scan time is in the order of minutes. This is sufficient for fairly gross, large scale cortical events that occur relatively slowly. Good results have been achieved by Ingvar and others (Ingvar, 1975, 1985; Roland, 1985) in localising cognitive functions in the brain by two-

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[2] Sterman, Harper, Hoppenbrouwers, McGinty, & Hodgman, 1977; Werner, Stockard & Bickford, 1977; Spehlmann, 1981; Sterman, McGinty, Harper, Hoppenbrouwers, & Hodgman, 1982; Jeannot, Fessard, Parain, Ensel, Le Dosseur, Brossard, Pierre, Devaux, & Thiebot, 1986.

[3] see Gilling & Brightwell (1982) for a review of these methods

and three-dimensional regional cerebral blood-flow measurements. However, these studies do not address on-going cortical events as they actually occur. It is usually not practical to reduce the scan time sufficiently to observe cortical events that come and go rapidly. Such events, which tend to have an inherently short latency period and poor persistence are typically those in the area of sensory perception. Events that imply rapid transit through sensory and perceptual pathways in the brain, such as evoked potentials, are too fleeting for most types of cerebral imaging.

A2.2.3. Traditionally, very rapid events in the cortex have been traced by means of their electrical discharges measured through the skull. This is electroencephalography or EEG. There is considerable debate within the electrophysiology literature as to what causes the surface EEG. It is not proposed to address this problem in any detail. However, Offenloch (1975) summarises the debate by saying:

> *"... the following hypothesis on the electrogenesis of the EEG: the surface macro-EEG potentials constitute the spatial summation of the extracellular potential fields of spontaneous and evoked membrane potential fluctuations of the individual neuronal - and possibly to some unknown extent also of glial - elements".* (page 82).

A2.2.4. In basic terms, scalp electrodes are placed according to an agreed montage (generally the international 10-20 system) and scalp potentials are recorded in real time. The technique is not without formidable technical problems. These include unfavourable signal-to-noise ratio, contamination by muscle movement (EMG), eye movement (EOG) and cardiac potentials (ECG). There is also the problem of generator (dipole) site and vector. This has recently been addressed by Pool, Aronofsky, Finitzo & Barr (1989, page 247), who concluded that these dipoles cannot be located.

A2.2.5. Another major problem is the sheer volume of analogue data produced. As Duffy, Lombroso & Burchfiel (1979) put it :

> *"... we propose that brain electrical activity presents not too little, but too much information to be easily grasped and assimilated by visual inspection alone."* (page 309).

EEG traces take many years of practice to interpret. Neuro-physiologists need to scan many metres of paper printouts of analogue EEG data to learn diagnosis. Even then, the probability of mis-diagnosis, and low inter-rater reliability is high. Recent progress in computerised manipulation of EEG has gone a long way to solving this problem. The work of Duffy *et al* (1979) and Etenvon[4] *et al* (1985) has shown that computer-based imaging of EEG is feasible and reliable. The technique of Brain Electrical Activity Mapping (BEAM) is the result. The aim of this method is to summarise the EEG signal in a visual fashion, such that changes become more apparent. Several BEAM systems are available commercially. The type used for the data reported in this thesis was a Neuroscience Series III, fitted with both floppy, and later, optical disk storage and a PtS data download system (see below). An illustration of this system is to be found in Plate 2, Chapter 4. An explanation of this method, applicable to adults and infants now follows.

A2.3.1. The BEAM technique is essentially an extension of traditional EEG methods and divides into three phases. These are (1) **acquisition**, (2) **transformation** (Fast Fourier Transform or FFT) and (3) **map generation**. It should be made clear that the interval between these phases is only 2.56 seconds, so the EEG display is virtually in real time.

(1) Acquisition phase

A2.3.2. This first phase is the most like traditional EEG. Up to thirty-two channels of data (28 EEG and up to four other physiological measures) are collected by a headcap (see Plate 1,

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[4]Etenvon, Gaches, Debouzy, Gueguen & Peron-Magnan (1985).

Chapter 4). This is a modification of the international 10-20 electrode configuration and provides much higher signal resolution. The data channels are amplified and filtered by an external pre-amplifier and routed to an analogue-to-digital converter where the data are sampled and digitised. The sampling rate is 256 Hz, which is governed by the 'rule of Nyquist'. This is a law in electronics which states that the sample rate must be at least twice the highest frequency in the signal. To ensure that this is possible, 'extraneous' high frequencies are filtered out electronically. Further technical details of operating mode whilst recording are summarised below.

Imager recording settings

Recording took place at 256 microvolt sensitivity. Some subjects with high amplitudes recorded at 512 microvolt sensitivity. Electronic filters on default settings (0.3 Hz [low] & 40 Hz [high]), notch (mains) filter off, autoscaling on, Delta waveband. On-screen event-marking by means of an electronic oscillator connected to a 'spare' channel and operated by a microswitch.

A2.3.3. Waveform acquisition was by means of one of three electrode headcaps, between 30 and 45 cm. in circumference. These had been designed from specifications developed by the experimenter and constructed by Meditec of Parma, Italy. The headcaps were constructed of a washable Lycra material and were slightly elasticated for closer fit. The 28 EEG electrodes were built into small plastic 'buttons'. The electrodes themselves were made of high-purity tin; electrical conductivity was achieved by a sodium chloride/potassium chloride (NaCl/KCl) based electrode gel (ECI Electrogel). The montage was configured in a variation of the international 10/20 convention; reference to linked ear lobes (see Plate 1, Chapter 4). The headcaps were secured to the subjects' head by a Velcro chin strap. Later subjects also had foam-backed self-adhesive inserts (Micropore adhesive) to electrodes FP1 and FP2.

(2) Transformation phase

A2.3.4. The digitised data are then **transformed** by means of an algorithm at the rate of 100 samples/second. This uses the Fast Fourier Transformation (FFT) to assign relative power within five frequency bands (delta, theta, alpha, beta 1 and beta 2). The reason for these five divisions is largely historical and has recently been challenged (Lorig & Schwartz, 1989). However, inaccessible software within the Brain Imager means that these divisions are unalterable with this system. The FFT is a mathematical method whereby very complex waves, comprising numerous frequency components, can be simplified. The original, complex wave is described in terms of a spectrum of frequencies - a series of simpler waves grouped into five bands. The product of the FFT is a series of coefficients which infer the relative power (the square of the amplitude) in five wavebands. This is a form of summary of the original EEG signal (Neuroscience Limited, personal communication, 1990).

A2.3.5. To perform the FFT, the digitised data are divided into 'bins' of 0.39 Hz width. This is the 'fundamental', or lowest resolvable frequency of the Fourier Transform and is the inverse of the time period, or epoch length. Hence, an epoch length of 2.56 seconds gives an inverse ($^1/_{256}$) of 0.39 Hz. The maximum resolvable frequency is given by the fundamental frequency multiplied by half the number of samples in an epoch. This equals (128 multiplied by 0.39) a figure just short of 50 Hz. The data are thus held in 128 frequency bins, each 0.39 Hz wide. Only the lowest 77 bins are further processed, because the Imager's mainly clinical use obviates the need for analysis of the highest cortical frequencies. Clinicians are generally only interested in EEG frequencies up to 30 Hz (Beta 2).

Map generation phase

A2.3.6. The results of the FFT analysis stage are then used in the **map generation phase** to construct topographic maps of power (power = amplitude squared) over the surface of the scalp, which is treated as a plane. These maps are then displayed on a high-resolution colour monitor, along with much other information. Power and distribution are represented as colour gradations, according to a

variable scale (see Plate 4, Chapter 4). Each of the 28 electrodes contributes one value to each of these maps. Colour between these points is generated by an interpolation algorithm, that works by trigonometrical means. These topographic maps can be decomposed into numbers for statistical analysis, with each minute of recording producing up to 3750 integers. Each map (it should be noted that, with this system, the terms 'map', 'frame' and 'epoch' can be used in an interchangeable fashion) is the end product of 2.56 seconds' worth of summated EEG data.

Data storage

A2.3.7. The problem of storage of this very large data set then arises. The system permits three forms of storage. The first, and most traditional of these is a paper printout in real time, whereby the EEG waveforms are routed to an external pen-writer. This output can then be examined in the traditional manner, usually by a trained neurophysiologist. Naturally, this system produces many sheets of recordings which require hours of trained analysis. The BEAM method is designed to obviate this necessity.

A2.3.8. The second form of storage is by means of a Winchester hard-disk drive. Data are held here on a temporary basis and then written to the floppy disk drive. A standard (720 kilobyte) 3.5" removable floppy disk is inserted in this drive and the data are stored on this disk in the form of brain maps. These can then be replayed as often as desired to permit examination of the cortical maps. These can be examined singly, or in sequence, in one frequency band or in all bands. The computer permits viewing of the maps by coronal projection, left or right saggital projection or left minus right to assess hemispheric differences. The only data not stored in this way are the actual EEG waveforms, due to storage constraints on floppy disks.

A2.3.9. The final storage method is by means of an optical disk. The optical disk drive employs a semiconductor laser to 'write' the EEG data and cortical maps on the optical media. The data (in the form of microscopic 'pits' in the plastic surface of the disk) can then be 'read' by the drive and reconstructed into cortical maps and

waveforms. This technology is known by the acronym **WORM** - for **W**rite **O**nce, **R**ead **M**any (times). The system allows replaying of maps and waveforms either by scrolling forwards or backwards, or paginating. The capacity of each disk is in excess of 200 megabytes of data. Once the data are stored, by whatever method, then analysis of the information is feasible. The system was designed principally for clinicians to facilitate diagnosis of cortical lesions and disease states such as epilepsy. For this reason, the visual appearance of the cortical maps is emphasised; using multiple views of the maps and various scrolling speeds. A high-resolution colour monitor is used as a display. Qualitative changes between maps are readily seen, because that is what the system was designed for. However, for analysis that extends beyond the qualitative dimension, system development was required. The following sections describe progress made to allow the necessary quantitative analysis of data.

Section 2: Data handling

A2.4.1. The Neuroscience Brain Imager has an in-built utility that allows statistical analysis of the brain maps. This is largely confined to non-parametric analyses and descriptive statistics such as individual and group means. In other words, an average map of one subject's cortical maps can be constructed and this used to compare with any other map. Similarly, a group of subjects can be constructed and any one group member compared with the group mean. This is useful in clinical terms for identifying normal, control patients and comparing them with a patient thought to be suffering from some pathology. A limited form of parametric analysis in the form of one-tailed 't test' can be performed. However, it is generally felt that this is insufficiently rigourous for analysis of data in psychological studies. Hence, some way of obtaining the raw data that go to compose the cortical maps was needed.

A2.4.2. The Imager allows these raw figures to obtained in the form of matrices. These comprise six columns, one for each EEG waveband and one for spectral map. There are thirty-two rows, comprising 28 electrodes and the four 'spare' channels. The latter channels are, by default, copies of four of the electrode channels and

are always ignored in any analysis because they are redundant. Each map, or 'frame' produces its own matrix, numbered zero to *n*, where *n* is the total number of frames recorded. Using the in-built statistics utility, these matrices can either be displayed on the Imager's monitor screen, or printed out on paper. Unfortunately, the original design of the software does not permit download to any electronic device, with the number matrices intact. Furthermore, the Imager software is not accessible, for two reasons. First, it is a non-standard form of disk operating system (DOS) and second, all the software is written on microchips. Hence a system had to be designed to permit data transfer, such that the matrices could be downloaded in a form legible by most computers - ASCII format.

A2.4.3. It was decided to try and intercept the output from the Imager as it was transmitting the number matrices to its line printer. A peripheral Ferranti micro-computer was obtained with this in mind. This idea was problematical in that the output is in parallel form, which cannot be readily transferred to a micro-computer. Therefore, a device was constructed to convert the parallel output to the printer into serial form that could be sent to a micro-computer. In practical terms this required considerable development, but a functioning device (known as a **Parallel-to-Serial Converter**, or **PtS** for short; see illustration in Plate 2, Chapter 4) was built. This device intercepted the output that would normally go to the Imager's printer and diverted it to the hard disk of the peripheral Ferranti micro-computer. In this way, the matrix format of the data was preserved intact. Data transfer was achieved by means of a modified form of the KERMIT terminal emulation/file transfer program. Speed of transfer was user-definable but generally ran at 2400 baud.

A2.4.4. Following data transfer, the matrices were checked with the originals produced by the Imager's statistics utility from the stored maps. This was to ensure that data corruption has not occurred in transit. Very large data sets could thus be handled (albeit rather slowly) and since each map or frame is identified by number, subject name and electrode position confusion between data sets was minimised. Files in excess of 100 kilobytes have been handled without any difficulty.

A2.4.5. The next stage of data transfer was to transmit the matrices to a mainframe computer that may be either local or remote. Since the data are stored as simple ASCII files, this process was relatively straightforward. From the Ferranti PC, the files were routed to a mainframe IBM 4381 computer, under the control of the KERMIT program mentioned above. This was achieved using the LASS (Local Area Switching System) lines within the University. Once transferred to the mainframe computer, the files were again checked for corruption. This process was necessary because corruption in transit, though unlikely, would lead to errors. In practice, a random sample of the files were cross-checked with the originals. The actual process was two-fold. First, the files were compared visually by calling up both originals and transmitted files onto adjacent screens. The second part of the process involved calling the files back from the mainframe computer onto the Ferranti PC and using the computer's operating system utilities (in this case, MS-DOS 'Compare' program) to ensure that the files were identical. Thus, the possibility of errors contaminating the analysis process was minimised.

Data analysis and interpretation

> *"An important and indispensable pre-requisite for CEAN* [computerised EEG analysis] *is the collaboration of experts on applied mathematics"* (Dolce & Decker, 1975)

A2.5.1. Very large data sets are usually problematical to handle. "Very large" depends, of course, on your definition. In comparison with a climatological study using atmospheric modelling, the data produced by the Brain Imager in the studies described below were relatively small-scale. However, in terms of psychology the data sets were large enough to be both unwieldy and hard to interpret. This section discusses the development of the analytical techniques that were used on the Imager data. It is essentially a discussion and evaluation of these methods. Furthermore, a discussion of what actually constituted the data sets will be addressed.

A2.5.2. One of the major problems of the Brain Imager data had always been how to display it. The highly visual nature of the topographic maps is at once a major strength and a major weakness of the BEAM technique. The strength lies in its ability to concisely summarise complex, real-time waveforms with a view to clinical diagnosis. This was the major reason for developing the technique (Duffy *et al*, 1979). However, this is also a weakness, because the seductive qualities of the map images mean that non-existent effects can be read into the maps. To put it another way, observer bias becomes a major problem when small changes are being searched for. Because the maps are visually attractive (by design), they can lead to errors of interpretation being inadvertently committed. This is because artifact looks very similar to signal.

A2.5.3. One way of differentiating the two is to review both maps and raw EEG waveforms simultaneously. In this way, perturbations in the waveforms due to artifact can be linked to the map appearances, as they occur. However, this is a cognitively demanding task, as experience had shown. The sheer volume of information produced on the Imager's monitor leads to rapid operator fatigue. This is a serious ergonomic problem, particularly in view of the amount of data produced by any one subject in a study. Furthermore, changes in map and waveform appearance are hard to assess because the information appears transiently, in the form in which it was acquired. It was not possible to easily view a sequence of recordings. Hence a method needed to be developed to overcome these problems.

A2.5.4. A technique was developed for graphical representation of the data sets. It had become clear that, although the colour topographical maps produced by the Imager were helpful in the initial screening process, they were hard to interpret. What was needed was another way of presenting the same data as a time-series, so that any effects due to odour presentation could be made clearer. To this end, a suite of FORTRAN programs were written to allow this. The final part of this Appendix contains documentation of these. What this software did was to read the raw data matrices and

convert them for graphical display, with the abscissa as time and the ordinate as amplitude in microvolts (see Figure 5, Chapter 4). This technique carried a number of advantages. First, any artifact electrodes became immediately obvious. Second, any synchronous cortical activity could be clearly related to an event such as odour presentation. Third, similarities or differences between subjects and within conditions became apparent. One other of this software suite could be used to present the data as separate 'mini-graphs', with one graph for each electrode. A number of subjects' data could be included on these if desired (see Figures 14 & 15).

A2.5.5. The major benefit of this form of data presentation was that artifact became very clear. As can be seen from Figure 5, Chapter 4 (q.v.), outlier electrode values are well demonstrated. Furthermore, as can be seen from Figure 16, the alternative hypothesis that the cortical response was simply due to an 'arousal phenomenon' is made less likely. This Figure should be compared with Figure 5 in Chapter 4. In Figure 5, but <u>not</u> in Figure 15, a degree of coherent activity, coincidental with stimulus presentation is visible. This appears to indicate that large areas of the cortex are responding simultaneously to a stimulus. As discussed in Chapter 4, the most parsimonious explanation for this is an orienting response. Because this technique represented the data as a time-series, it proved an efficient summary of the data. All that was needed was an inferential statistical technique, to assess the null hypothesis. In practice, this proved to be very difficult, probably because of the multivariate nature of the data, as well as the wide within- and between-subjects variability. This latter problem ensured that assumptions about a normal data distribution were unlikely to be valid. All these difficulties are, of course, common to all types of quantified EEG research. They have been summarised to some extent in a paper by Rappelsberger & Petsche (1988).

A2.5.6. These workers considered the difficulties of statistically analysing EEG values that were somewhat similar to those described in this thesis. However, it should be made clear that Rappelsberger & Petsche were testing adults in purely cognitive tasks, using a different imaging system. Nonetheless, they reach the

FIGURE 14:

GRAPHIC REPRESENTATION OF BRAIN IMAGER DATA. THE ABSCISSA IS TIME IN 'FRAMES' (ONE FRAME = 2.56 SECONDS), FROM ZERO TO 30. THE ORDINATE IS AMPLITUDE IN MICROVOLTS. DATA FROM ONE SUBJECT, ODOUR 2 (CHOCOLATE PUDDING). THE ODOUR WAS PRESENTED AT A POINT ONE THIRD ALONG THE TIME AXIS. EACH SMALL GRAPH REPRESENTS OUTPUT FROM A SINGLE ELECTRODE.

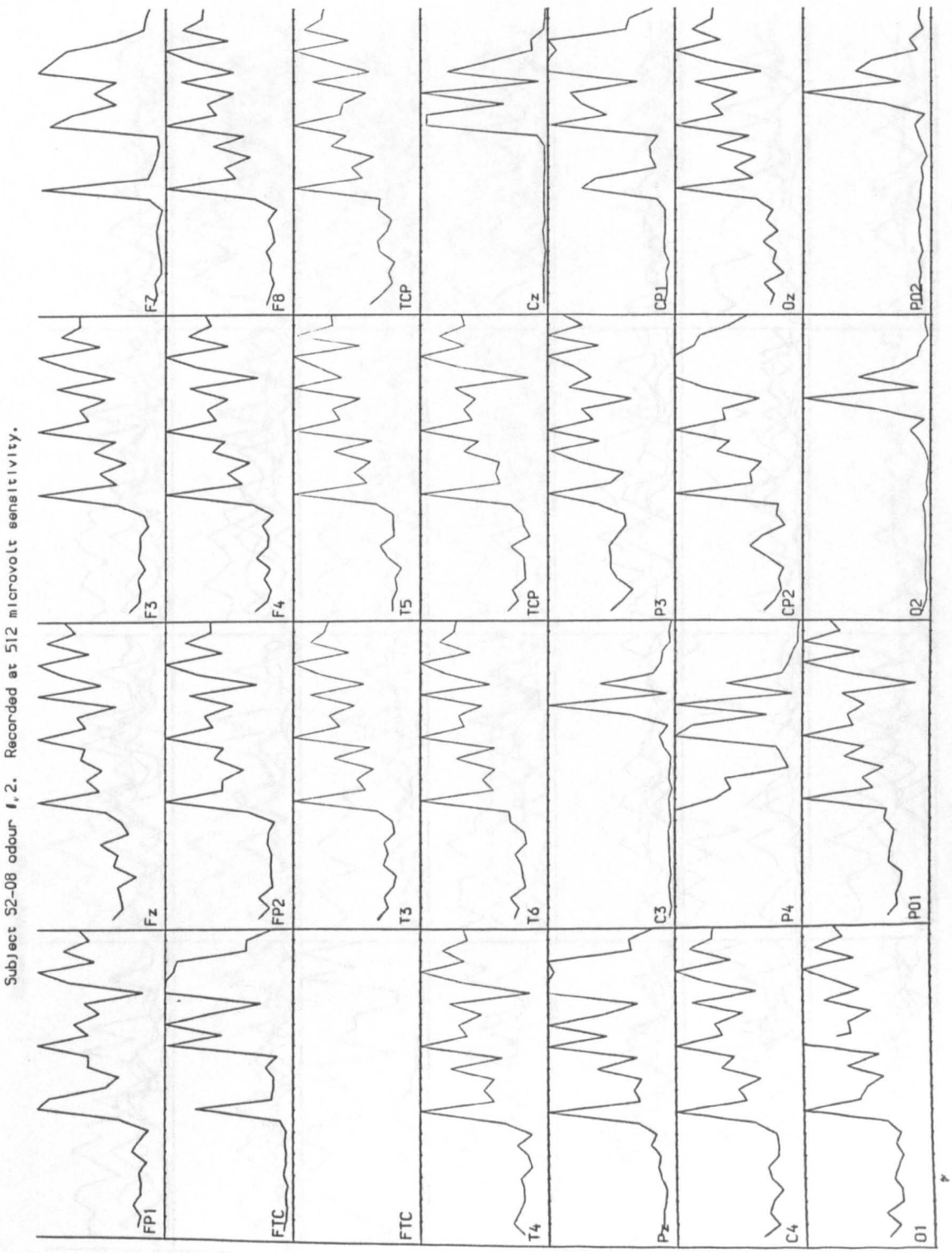


FIGURE 15:

COMBINED DATA FROM FOUR SUBJECTS, PRESENTED WITH ODOUR 3 (BEEF DINNER). AXES AND OTHER CONDITIONS ARE THE SAME AS FIGURE 14.

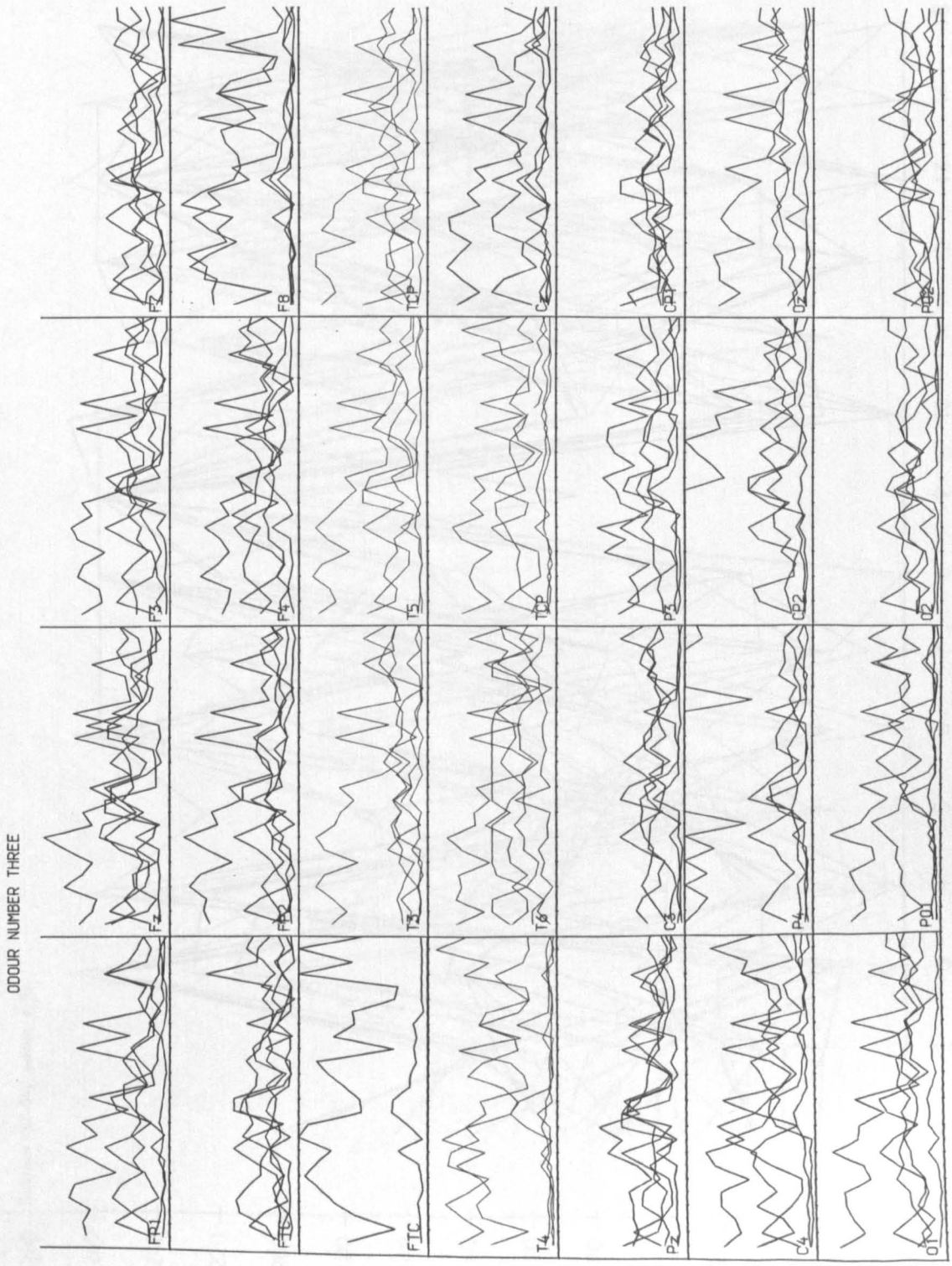
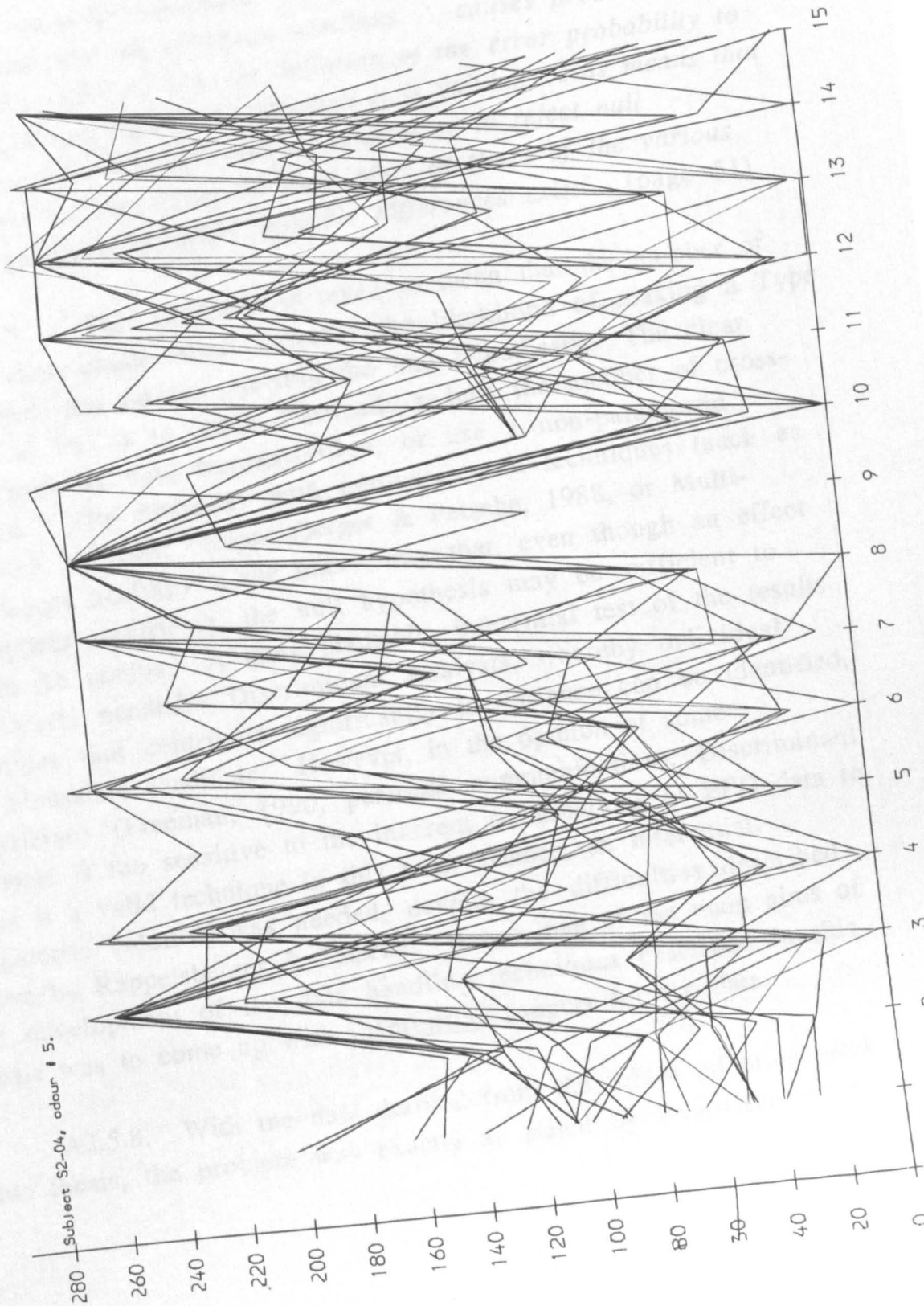


FIGURE 16:

DATA FROM ONE SUBJECT IN RESPONSE TO STIMULUS 5 (TONE). NO COHERENT RESPONSES SIMILAR TO THOSE FOLLOWING ODOUR PRESENTATION CAN BE SEEN.



conclusion that the quantitative analysis of multivariate EEG data is highly problematical. This is due to the reasons described above, as well as the problem of the number of possible variables that might influence the outcome of the analysis. Rappelsberger & Petsche summarised it thus:

> *"From the statistical point of view the significance testing of many variables like theta, alpha and beta power and at many locations like 19 electrode positions... causes problems because of the p-inflation (i.e. the inflation of the error probability to reject a null hypothesis although it is valid.... This means that the statistics are not used to confirm or to reject null hypotheses but rather to yield hints at those of the various comparisons for which possible differences exist"* (page 51).

A2.5.7. This statement is taken to mean that the number of possible cross-comparisons increases the likelihood of making a Type I error and thus falsely rejecting the null hypothesis. The clear solution to this is to either drastically reduce the number of cross-comparisons by data summarisation, or use a non-parametric technique. The difficulty with non-parametric techniques (such as Coherence Analysis: Rappelsberger & Petsche, 1988, or Multi-Dimensional Scaling) is the uncertainty that, even though an effect may appear significant, the null hypothesis may be sufficient to explain the results. A more plausible, inferential test of the results was clearly needed. Discriminant Analysis, whereby individual electrodes that contribute significantly to variance can be identified, was a possible candidate. However, in the opinion of some statisticians (Freeman, 1990, personal communication), Discriminant Analysis is too sensitive to the inherent perturbations of EEG data to make it a valid technique in this area. Hence, an inferential, parametric solution was needed, despite the difficulties described above by Rappelsberger & Petsche. Hence, one of the main aims of the development of the data handling techniques described in this thesis was to come up with <u>inferential</u> support for the data.

A2.5.8. With the data derived from the infant olfaction work in this thesis, the problem was exactly as stated by Rappelsberger &

Petsche, only more so. With 28 electrode positions, 5 wavebands, up to 7 conditions, and up to 30 data sets ('frames') for each subject in each condition, the scale of the analysis problem can be seen even with a relatively small sample of subjects. One way round this was to reduce the data set to one waveband. As described elsewhere in this thesis, most infant cortical activity seemed to occur in the delta frequency range (up to 4 Hz). The other wavebands were accordingly not examined for analysis purposes.

A2.5.9. To this end, further software was written to permit analysis. This technique was available to examine data produced by the second and final Imager study, described in Chapter 4. This involved the use of the Low Odour Room (LOR). The MC2 software addressed the variances of the data. The rationale behind using using this summary statistic was as follows. Because EEG data tends to vary on a number of dimensions (phase, negative or positive wave direction, amplitude) reduction of data to a mean value is of low value. This is because, at any one epoch ('frame'), some electrode values are increasing in amplitude, some are decreasing, and a few are showing little variation. An example of this is clearly shown in Figure 5, as described above. Averaging them all together into a mean value does not reflect this variability. Such data are not well described by reduction to a mean value (Ehrenburg, 1982, page 23). A slightly better, but still less than perfect descriptive summary statistic would be the variance. Because this describes the scatter of the data around any putative mean, it gives a slightly more realistic view of how the data are behaving at any given time.

A2.5.10. With the data obtained in the LOR experiment, what was observed (using the MCS series of software to re-represent the data graphically) was some apparent synchronisation of EEG at the time when the odour was presented. It was felt that computing the data variance would hence show a lower value than during the 'baseline' EEG. Therefore, the hypothesis was that variances for the data prior to odour presentation would be fairly high. Then, if any EEG synchronisation occurred at the point of odour presentation (frame 10), the variances would be lower. The MC2 program was written to test this hypothesis.

A2.5.11. The program permitted artifact deletion, in a very crude form. A value could be defined (following manual inspection of the data sets) that represented artifact. These values would then be recoded as 'missing data' so as not to unduly influence the variance calculation. The program then calculated variances for all electrodes for every subject included in the input file. An option was provided for excluding subjects with apparently anomalous data. These variances were then ranked in ascending order (1 to 28), with the option of choosing any one of these for analysis. Results from this are given in Chapter 4.

Documentation for MCS programs for Brain Imager data handling on IBM mainframe computer

A2.6.1. This final section of this Appendix includes details of the software written to handle the data produced by the Neuroscience Series III Brain Imager used in the studies described in this thesis. Programs were defined by the author and written (in REXX, FORTRAN and PASCAL) by Mr. Keith Halstead. The programs are given in full at the end of this section.

MCS1 software

A2.6.2. This program reads Imager files entire, providing the necessary header information is present. The first data matrix ('frame') is always deleted. The program can only read one data file. User-definable options are: highest frame number to be included, waveband (five classic bands), and odour number (up to five). The file is then read into the GHOST graphics program on SKY to plot the result as an A3 size colour graph. The abscissa is time, in frames (hence 2.56 second epochs); the ordinate is amplitude in microvolts. The plot is colour coded as follows: **black** (frontal electrodes), **red** (temporal electrodes), **green** (parietal electrodes) and **blue** (occipital electrodes). The plot also includes the header information in terms of subject code and odour number.

MCS2 software

A2.6.3. This is essentially identical with MCS1, but produces multiple plots - one for each electrode. Same options, constraints and colour codes as MCS1 apply.

MCS3 software

A2.6.4. This program reads Imager data files into a format addressable by SAS™ only. It is then used to invoke a number of SAS™ routines (including a program called MCS3 SAS will correlate electrodes in one file).

MCS4 software

A2.6.5. This program will read in up to 10 Imager raw data files and display them with a user-definable heading in a similar fashion to MCS2. Hence each separate electrode can be plotted with data for up to 10 subjects in one odour condition. The degree of coherence between subjects can then be visually assessed for that odour.

MCS5 software

A2.6.6. This program combines many of the features of the previous versions as well as permitting a range of analyses, provided by NAG™ software. Again, up to 10 files can be concatenated, and the resulting plots given a title by the user. The usual range of options are provided (subject numbers, waveforms and odour numbers). Additionally, the user can define whether the raw values are used for the computation, or the differences between adjacent values in the raw data matrices. This latter option allows the general direction of the values to be assessed, as with the raw values, values in one subject may be rising at the same time as others are falling. The program then allows display of the means of the data, the variance of the data, auto-correlations and a form of Eigen-value. These values are all displayed on 'mini-graphs' as described above.

MCS6 software

A2.6.7. This program is designed for Discriminant Analysis, using BMDP7M™ software. As with the other programs in this suite, it reads Imager data files and permits user options. In this case, it

permits analysis of a number of odour conditions in any one data set. This program also allows the maximum 'frame' number to be specified. This permits analysis of a smaller or larger time period. The Discriminant Analysis will also address a 'window' of frames within the larger set already specified. The resulting output from the program is contained in a very comprehensive listing file.

MC2 software

A2.6.8. This is the final and most elaborate program in the suite. The aim is to read the data files and calculate variances for all electrodes for all subjects in each odour condition. The program was written in PASCAL. All data files had to go through a data-reduction process, whereby only the waveband of interest was used. Hence, only those columns containing Delta were used. All subjects' data could then be appended into master files, containing Delta values for all subjects in each odour condition. These master files constituted the input for MC2.

A2.6.9. The program permits artifact identification and deletion according to user-definable criteria. Any cutoff point for data judged due to artifact can be selected. A typical dialogue with this program is as follows:

give the cut-off values for artifacts:	**3 5 0**
give the start and end of the first window:	**5 10**
give the start and end of the second window:	**11 16**
give the low index for the percentile:	**1 4**
give the high index for the percentile:	**1 5**
give the output file name:	**ODOUR2 EG1** (example)
give the heading:	**RESULTS FOR ODOUR2**
give the data file name:	**S2ODOUR1 DELTA** (the output file from the data-reduction program mentioned above)

At this stage the name of each individual on S2ODOUR1 DELTA is typed, and the user invited to type 1 (to include the individual) or 0 (to exclude). At the end of the files the user is asked to type 1 for

another file, or 0 to terminate. The values 14 and 15 for the percentile in effect specify the median, as the mean of the 14th and 15th values will be used. Following the order statistics all the variances are printed.

PROGRAMS CORRESPONDING TO THE DESCRIPTIONS GIVEN ABOVE

MCS1 EXEC.

A2.7.1. "This is an exec to read Imager data files into a graphics routine to display data as a series of curves. The raw data files MUST contain header info for each set of matrices. This must run thus: "Subject S1-01 (for example) odour # n", where n = the condition. The first matrix (frame 0) is always erased. The resulting plot, which is called DEFAULT PLOT, can be printed on the HP plotter. The x axis will be frames 1-n and the y axis amplitude in microvolts. The colour codes are BLACK for the frontal electrodes, RED for the temporal ones, GREEN for the parietal and BLUE for occipital electrodes".

```
trace  n
say 'Give the name of the data file'
pull fn ft fm .
if ft='' then ft='DATA'
fil=fn  ft
'LISTFILE' fil '( DATE LIFO'
pull  ll
parse var ll . . . ff nn .
if ff<>'F' | nn<>80 then do
   'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
   'QERASE' fil
   'RENAME' fn 'ZZZZ A' fil 'A'
end
'QERASE DEFAULT GRID'
'FILEDEF 7 DISK ' fn ft '*'
'RUNF MCS1 GRID'
exit
```

MCS2 EXEC

A2.7.2. "This is an exec to read Imager data files into a graphics routine for plotting onscreen, or to the HP plotter. The same header information needed for MCS1 EXEC (which see) is needed for this one also. The same constraints apply. However, this exec produces multiple plots - one for each electrode. Colour codes are the same as for MCS1 EXEC".

```
trace  n
say 'Give the name of the data file'
pull fn ft fm .
if ft='' then ft='DATA'
fil=fn  ft
'LISTFILE' fil '( DATE LIFO'
pull  ll
parse var ll . . . ff nn .
if ff<>'F' | nn<>80 then do
   'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
   'QERASE' fil
   'RENAME' fn 'ZZZZ A' fil 'A'
end
'QERASE DEFAULT GRID'
'FILEDEF 7 DISK ' fn ft '*'
'RUNF MCS2 GRID'
exit
```

MCS3 EXEC

A2.7.3. "This is an exec to read Imager data into the SAS system for analysis. No graphics are produced. Once the output file has been produced by this exec, SAS can be invoked. The control program is called MCS3 SAS (it is user definable). To run it enter 'SAS MCS3'".

```
trace n
say 'Give the name of the input data file'
pull fn ft fm .
if ft='' then ft='DATA'
say 'Give the name of the output data file'
pull fno fto fmo .
if fto='' then fto='SASDATA'
filo=fno fto fmo
'QERASE' filo
fil=fn ft fm
'LISTFILE' fil '( DATE LIFO'
pull ll
parse var ll . . . ff nn .
if ff<>'F' | nn<>80 then do
  'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
  'QERASE' fil
  'RENAME' fn 'ZZZZ A' fil 'A'
end
'FILEDEF 7 DISK ' fn ft '*'
'FILEDEF 8 DISK ' filo
'RUNF MCS3 '
exit
```

MCS4 EXEC

A2.7.4. "This program is a development of the previous graphics programs".

```
trace n
say 'Give the heading'
pull heading
say 'How many data files '
pull filnum
if filnum>10 then do
   say 'current limit of 10 files'
  exit
end
fifn=6
do fi=1 to filnum
   say 'Give the name of the data file'
   pull fn ft fm .
   if ft='' then ft='DATA'
   fil=fn ft
  'LISTFILE' fil '( DATE LIFO'
   pull ll
   parse var ll . . . ff nn .
   if ff<>'F' | nn<>80 then do
```

```
    'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
    'QERASE' fil
     'RENAME' fn 'ZZZZ A' fil 'A'
   end
    fifn=fifn+1
   'FILEDEF' fifn 'DISK' fn ft '*'
end
'QERASE MCS4 DATA A'
v=filnum
'EXECIO 1 DISKW MCS4 DATA A  1 F 80 ( VAR V'
'EXECIO 1 DISKW MCS4 DATA A  2 F 80 ( VAR HEADING'
'QERASE DEFAULT GRID'
'FILEDEF 2 DISK MCS4 DATA A'
'RUNF MCS4 GRID'
exit
```

MCS5 EXEC

A2.7.5. "This program is a development of the previous graphics programs".

```
trace  n
say  'Give  the  heading'
pull   heading
say  'How  many  data  files  '
pull   filnum
if  filnum>10  then  do
    say  'current  limit  of  10  files'
   exit
end
fifn=6
do  fi=1  to  filnum
    say  'Give  the  name  of  the  data  file'
   pull fn ft fm .
   if ft='' then ft='DATA'
   fil=fn ft
  'LISTFILE' fil '( DATE LIFO'
   pull ll
   parse var ll . . . ff nn .
   if ff<>'F' | nn<>80 then do
    'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
    'QERASE' fil
     'RENAME' fn 'ZZZZ A' fil 'A'
   end
    fifn=fifn+1
   'FILEDEF' fifn 'DISK' fn ft '*'
end
'QERASE MCS5 DATA A'
v=filnum
'EXECIO 1 DISKW MCS5 DATA A  1 F 80 ( VAR V'
'EXECIO 1 DISKW MCS5 DATA A  2 F 80 ( VAR HEADING'
'QERASE DEFAULT GRID'
'FILEDEF 2 DISK MCS5 DATA A'
'RUNF MCS5 GRID NAG'
exit
```

MCS6 EXEC

A2.7.6. "This program is a development of the previous graphics programs".

```
trace n
arg fn ft fm
a.1 ='FP1 '
a.2 ='Fz  '
a.3 ='Cz  '
a.4 ='Pz  '
a.5 ='Oz  '
a.6 ='F3  '
a.7 ='C3  '
a.8 ='P3  '
a.9 ='O1  '
a.10='F7  '
a.11='T3  '
a.12='T5  '
a.13='FTC1'
a.14='TCP1'
a.15='CP1 '
a.16='PO1 '
a.17='FP2 '
a.18='F4  '
a.19='C4  '
a.20='P4  '
a.21='O2  '
a.22='F8  '
a.23='T4  '
a.24='T6  '
a.25='FTC2'
a.26='TCP2'
a.27='CP2 '
a.28='PO2 '
if fn='' then fff=''
else do
   fff=fn
   if ft='' then fff=fff 'DATA'
   else fff=fff ft
   if fm='' then fff=fff 'A'
   else fff=fff fm
   say 'reading file ' fff
end
prompt=1
if fff<>'' then prompt=0
if fff<>'' then do
  'EXECIO 1 DISKR' fff '( VAR HEADING'
  'EXECIO 1 DISKR' fff '( VAR FILNUM'
   do i=1 to filnum
     'EXECIO 1 DISKR' fff '( FIFO'
      myrc=rc
   end
  'EXECIO 1 DISKR' fff '( VAR ODLIST'
```

```
  'EXECIO 1 DISKR' fff '( VAR ELLIST'
end
if prompt then say 'Give the heading'
if prompt then pull heading
if prompt then say 'How many data files '
if prompt then pull filnum
fifn=6
do fi=1 to filnum
    if prompt then say 'Give the name of the data file'
   pull fn ft fm .
   if ft='' then ft='DATA'
   fil=fn ft
  'LISTFILE' fil '( DATE LIFO'
   pull ll
   say ll
   parse var ll . . . ff nn .
   if ff<>'F' | nn<>80 then do
    'COPYFILE' fil 'A' fn 'ZZZZ A ( RECFM F LRECL 80'
    'QERASE' fil
     'RENAME' fn 'ZZZZ A' fil 'A'
   end
    fifn=fifn+1
   'FILEDEF' fifn 'DISK' fn ft '*'
end
if prompt then do
   say 'Give a list of odours'
   pull odlist
end
odcount=0
ddd=strip(odlist)
do while ddd<>''
   parse var ddd p1 p2
    p1=strip(p1)
    ddd=strip(p2)
   if p1<>'' then do
       odcount=odcount+1
       odlist.odcount=p1
   end
end
odent=odcount
do i=1 to odcount
   odent=odent  odlist.i
end
if prompt then do
   say 'Give a list of electrodes ( 1 to 5 for d t a b1 b2 )'
   pull ellist
end
elcount=0
ddd=strip(ellist)
ellist.=0
do while ddd<>''
   parse var ddd p1 p2
    p1=strip(p1)
    ddd=strip(p2)
   if p1<>'' then do
       elcount=elcount+1
     ellist.p1=1
   end
```

```
end
elent=ellist.1 ellist.2 ellist.3 ellist.4 ellist.5
say elent
'QERASE MCS6 DATA A'
v=filnum
'EXECIO 1 DISKW MCS6 DATA A 1 F 80 ( VAR V'
'EXECIO 1 DISKW MCS6 DATA A 2 F 80 ( VAR ODENT'
'EXECIO 1 DISKW MCS6 DATA A 3 F 80 ( VAR ELENT'
'FINIS MCS6 DATA A'
'QERASE DEFAULT GRID'
'FILEDEF 1 DISK MCS6 RAW'
'FILEDEF 2 DISK MCS6 DATA A'
'RUNF MCS6'
'QERASE MCS6 DATA A'
'QERASE MCS6 BMDP A'
if length(heading)>60 then heading=substr(heading,1,60)
v="/PROBLEM TITLE='"heading"'."
'EXECIO 1 DISKW MCS6 BMDP A 1 F 80 ( VAR V'
v='/INPUT VARIABLES ARE '1+elcount*28'.'
'EXECIO 1 DISKW MCS6 BMDP A 2 F 80 ( VAR V'
n=elcount
v=" FORMAT IS '("n+1"F5.0/26(X5,"n"F5.0/),X5,"n"f5.0)'."
'EXECIO 1 DISKW MCS6 BMDP A 3 F 80 ( VAR V'
v=" UNIT=10."
'EXECIO 1 DISKW MCS6 BMDP A 4 F 80 ( VAR V'
v=" GROUPS="odcount+1'.'
'EXECIO 1 DISKW MCS6 BMDP A 5 F 80 ( VAR V'
v="/VARIABLE NAMES ARE CODN,"
'EXECIO 1 DISKW MCS6 BMDP A 6 F 80 ( VAR V'
lin=6
do i=1 to 28
   lin=lin+1
  aa=a.i
  v="
   if ellist.1 then do
     if v=" then v="
    else
    v=v','
    v=v||'DD'aa
  end
   if ellist.2 then do
     if v=" then v="
    else
    v=v','
    v=v||'TT'aa
  end
   if ellist.3 then do
     if v=" then v="
    else
    v=v','
    v=v||'AA'aa
  end
   if ellist.4 then do
     if v=" then v="
    else
    v=v','
    v=v||'B1'aa
  end
```

```
    if ellist.5 then do
      if v='' then v=''
     else
     v=v','
      v=v||'B2'aa
   end
    if i=28 then v=v'.'
    else v=v','
  'EXECIO 1 DISKW MCS6 BMDP A 'lin' F 80 ( VAR V'
end
lin=lin+1
v="USE = 1 TO "1+elcount*28"."
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
lin=lin+1
v=" GROUPING = CODN."
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
v="/GROUPING   CODES("odcount+1") ARE 0,"
do i=1 to odcount
   v=v odlist.i
    if i<odcount then v=v','
   else v=v'.'
end
lin=lin+1
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
v="/DISCRIMINANT ENTER=1.9,1.9 ."
lin=lin+1
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
v=" REMOVE=1.6,1.6 ."
lin=lin+1
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
v="/END "
lin=lin+1
'EXECIO 1 DISKW MCS6 BMDP A' lin 'F 80 ( VAR V'
'FINIS * * '
'FI 10 DISK MCS6 RAW A'
'BMDP7M < MCS6 > MCS6L'
exit
```

APPENDIX 3
DETAILS OF THE PREPARATORY PHASE FOR THE EMPIRICAL WORK CARRIED OUT FOR THIS THESIS.

Introduction

A3.1.1. This Appendix provides further details of the **Preparatory Phase** for the empirical work described in Chapter 4 of this thesis. Topics in this Appendix are cited where appropriate, in Chapter 4. This Appendix is divided into three sections. The first of these sections gives full details of the recruitment system which had to be set up in order to ensure a steady supply of babies for testing. The second section deals with some of the problems associated with adapting the BEAM technique to infants. The third and final section covers the rationale behind the choice of stimuli.

Section 1: Subject recruitment

A3.1.2. As with all human testing, priority was given to the ethical considerations involved. During the preparatory phase described in Chapter 4, the ethical committee set up by the local Health Authority was approached with an experimental proposal. This committee comprised senior physicians, surgeons and obstetricians. Approval was given by the committee without any modifications to the proposal. Approval was also sought from an ethical committee set up by the Department of Psychology, University of Warwick. This committee consisted of senior academics, and sanction was also given for the studies. Permission under the Data Protection Act, regarding data confidentiality, was also granted.

A3.1.3. Following experience with subject recruitment during preliminary work in the USA, it was decided to use direct-mailing with a standard letter. This system had worked very successfully with American parents and it was hoped that a similar result was possible in the UK. The letter is reproduced below.

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Dear Parents

Congratulations on the birth of your baby. This letter is to introduce you to some important research we are undertaking on young infants and to ask if you would consider taking part with your baby. We have been supplied with your name and address **only** by the Coventry Health Authority from birth registration information and have undertaken to keep this confidential.

We are psychologists working in the Department of Psychology, University of Warwick who are interested in how the sense of smell of babies develops. Smell is an important but neglected part of our lives. To imagine life without it we have only to think back to our last heavy cold when a blocked nose meant that not only was the world around us drab without smell, but food was tasteless and unappetising.

That is one side of the problem, but how do babies learn about the smell of things? Well, a research study has shown that in a few days from birth a baby learns to recognise the smell of his/her mother and will then reject a strange mother. This result shows that babies start to learn about the smells around them at a very young age. What we are doing in our research is to explore how smell, or to use the technical term, olfactory learning comes about. This is of particular importance for understanding food preferences and how babies develop "food-fads".

We are asking you to allow us to test your baby once only at about twelve weeks of age. The test is harmless and has been approved by a team of doctors in the Coventry Health Authority who have been set up to vet proposed studies. Your baby will be required to wear a cap which contains tiny sensors. These allow us to record the normal brain waves whilst your baby is smelling pleasant food odours. We may also wish to record your baby's breathing pattern at the same time. During the whole test the baby will be sitting on your lap and under your control. The person making the test is qualified as both a nurse and a psychologist.

The setting up and testing will take about one hour. After the test we will give you a Polaroid photograph of you and your baby in the testing situation and a coloured map showing the brain activity of your child. You will also learn about the neglected sense of smell and some of its effects on our lives.

If you would like to arrange for us to test your baby or, alternatively, obtain further information please telephone us at the University of Warwick, **Coventry 523523 ext.2774.** An answering machine is in use out of office hours if you wish to call then.

Yours sincerely,

Martin Kendal-Reed & Steve Van Toller

A3.1.4. This direct-mail system was very time-consuming, involving at least two full days per month, to mail up to 400 letters. In the initial phases, the letters had hand-written addresses. This was later superseded by labels prepared by computer. Once the address lists were received each month from the Health Authority, they were cross-checked with child mortality data to try and preclude any distressing errors during mailing. The printed lists were then entered into the University of Warwick's mainframe computer by data-entry staff. Following checking, the printed lists were then shredded, for security reasons. Once they had been entered onto the computer, access was restricted to the experimenter only. This was ensured by regular changes of username password to prevent illicit access by 'computer hackers'. Because of the security requirements implied by the Data Protection Act, these lists were erased from the computer system at the end of each testing period.

A3.1.5. Software specially developed for this research programme then produced address labels for direct-mailing of the parents. This recruitment system was successful, in that approximately 5-10% of those mailed contacted the laboratory to express interest. Nearly all of those who did so were subsequently recruited. The testing period extended from early August 1988, to the middle of March, 1990. During this period, 268 subjects were recruited and testing attempted in all cases.

A3.1.6. As part of a public-relations effort designed to increase public awareness of the research, the experimenter also visited a local maternity hospital four times a month. With the permission of the nursing and medical staff, mothers visiting the ante-natal clinic at a late stage of pregnancy were interviewed. Only those who had previously expressed an interest in infant research were seen. The mothers were told about the infant olfaction research and asked if they would consider participating at a later stage. The opportunity was also taken to increase their awareness of the importance of olfaction in infancy. Interestingly, many of the mothers had already heard of the work of researchers such as Macfarlane, Schaal and Porter. However, after a year of these visits it was felt that they did

not serve to significantly increase the number of subjects recruited. The maternity hospital visits were therefore discontinued. Another recruitment system was also tried. A nearby neighbourhood Health Centre was contacted to ask whether the medical staff would permit similar visits. This was agreed with enthusiasm by the doctors concerned and about ten visits were made over a period of six months. However, the visits were discontinued for the same reasons as above.

A3.1.7. The question arose as to how parents would reach the laboratory for their testing appointment. Although parents representing most socio-economic groups were recruited, the majority were car-owners. For these parents, special parking arrangements were made, to allow the easiest possible access to the laboratory. When the mothers telephoned the laboratory to make their appointments, it was explained that reserved parking less than a hundred metres from the laboratory had been arranged. This was particularly beneficial during inclement weather. Mothers were requested to report to the main entrance to the University on arrival. They were then directed by Security staff to the reserved parking spot near the testing laboratory. Those arriving by public transport were met by the experimenter at a nearby bus stop. It should be made clear that no reimbursement of travel expenses was offered due to financial constraints.

A3.2.1. It was decided at an early stage only to test infants at three months of age, for the following reasons. Firstly, the experimenter had considerable experience with this age-group derived from the preliminary work in the USA, summarised above. Secondly, it was felt that this age-group was a reasonable compromise between the need to test very young infants, in order to test the model discussed in Chapter 3, and pragmatic reasons. As most infant experimenters know, babies younger than about ten weeks spend most of their time asleep, rendering them difficult to test. Older babies tend to be more alert.

A3.2.2. Thirdly, many of the babies in the test age-group would have begun weaning and hence had some food odour

experience. This was important for the reasons described below in Section 3. Fourthly, the age of weaning is a time of transition from long-range odour experience to close-range experience and learning. Because of this, it was hoped that clearer, more dramatic cortical responses might be elicited because of the growth in cortical interconnections that might be expected to accompany this transition. Lastly, these slightly older babies would be of a size whereby headcaps would be technically easier to manufacture and fit on the subjects.

A3.2.3. As can be seen from the standard letter, parents were invited to telephone the laboratory for further information. If they so wished, a mutually convenient appointment time was then agreed. This was arranged so as to coincide as far as possible with the child's 'three month birthday'. Mothers were also encouraged to arrange appointments at either 10 a.m. or 2 p.m., when many infants have been fed. These times were chosen from previous experience which showed at what time infants would be at their most cooperative.

Section 2: BEAM and human infants

A3.3.1. All subjects were tested with a Neuroscience Series III Brain Imager, as described in the Technical Appendix (Appendix 2). Experience with Brain Imaging was gained during preliminary, adult work (Van Toller, Kendal-Reed, & Sleight, 1989; Van Toller & Kendal-Reed, 1990; Van Toller *et al*, 1990). Testing of adults was usually a two person operation. One would operate the Brain Imager, and the other would present the stimuli. This situation was not possible for infant testing, so the Imager had to be modified to allow solo operation. This was just part of the very considerable preliminary work which was needed in order to produce usable data from infant subjects. The Brain Imager came supplied with a small-size headcap that was meant to be used with children. However, it rapidly became apparent that it would be too large for most three month old babies.

A3.3.2. For this reason, three special infant headcaps were commissioned from a European manufacturer (Meditec/Henley's Medical). An example can be seen in Plate 1. This involved

protracted specification and design development. It was decided from anthropometric data that the maximum head circumference in the sample would be less than 45 cm. Hence headcaps were eventually constructed to fit infants with a head circumference (nasion to inion measurement) of between 30 and 40 cm. The headcaps were made of a lightweight, slightly elastic nylon material. Specifications are found in the Technical Appendix (Appendix 2). The criteria for good fit were necessarily stringent. Subjects who did not tolerate the headcap, even though fitting had begun, were deemed unsuitable for the BEAM study. The reason for this was as follows. Because headcap intolerance was usually manifested by crying, the subjects perforce did not, and would not have fulfilled the criteria of 'settled'. Their EEG data, even had the recording gone ahead, would have been highly erratic and probably much contaminated by movement. It would also have been unfair to the mothers, as well as ethically dubious, to continue when it was plain that the child was unhappy with the procedure.

A3.3.3. As mentioned in the Technical Appendix (Appendix 2), EEG measurement requires a source of electrical reference. In the BEAM technique, this is usually provided by linked earlobe electrodes, attached by means of spring-clips. The type that had been supplied with the headcaps were found to be unsatisfactory for infants, so a modification was made to allow use of the regular adult type. In general, these appeared to work well, though they had to be checked between trials in case they slipped off. Electrode gel was inserted into each electrode by a blunted needle and syringe, described below.

A3.3.4. The original method of securing the headcap by means of a chest-band, as used in adults, was found to be inappropriate in babies and was abandoned. A fixation method with a Velcro chin-strap was developed. The advantage of this was that it permitted a more snug fit and did not seem to distress subjects at all. Nonetheless, as infant skulls have a long ventral-dorsal axis, the headcap was prone to slide backwards. In practice this usually meant that the frontal electrodes were positioned more posteriorly than would be the case in adults. Subsequent experience showed

that it was best to use self-adhesive foam pads on the frontal electrodes to try and avoid this problem. This technique was used in subjects tested in Experimental Study 2.

A3.3.5. If the headcap fit was dubious, then it was not used. The reason for this was that, even with a good headcap fit, baby EEG is prone to poor signal-to-noise ratio, as stated by Kagawa (1962). A poor headcap fit, with uncertain electrode contact, would have severely worsened this problem. Hence, it was decided from an early stage to be conservative in deciding how good the headcap fit was. This inevitably meant that most subjects were deemed unsuitable. Of the 254 subjects recruited, only about 10% were suitable for BEAM testing. This was partly the result of having a small range of three headcaps.

A3.3.6. For unsuitable subjects, the Respiratory Plethysmography (RP) technique was substituted, if the mother was agreeable to continuing testing. The purpose was to avoid 'wasting' recruited infants. This method was developed from one used during preliminary studies in the USA (see Appendix 1) and is described in the latter part of Chapter 4, as Experimental Study 3.

A3.3.7. As described in the Technical Appendix (Appendix 2), electrical impedance should be as low as possible to improve the signal-to-noise ratio in EEG recording. For this reason, no subject was tested who suffered from any kind of dermatological problem that might create impedance, such as 'cradle-cap'. Babies have naturally oily scalps that produce high impedances. During the very early stages of pilot work, several methods of reducing the greasiness of the scalp were tried, in order to lower skin impedance. These methods included the use of methylated or surgical spirit. These were found to be too odorous and would have constituted a confounding variable. Later subjects received absolute alcohol (95% pure), which virtually odourless when permitted to dry off. This was gently wiped over the scalp surface with a piece of cotton wool and allowed to dry. It was hoped that reducing the amount of skin grease on the scalp with alcohol might lower impedance from the very high levels found on the untreated scalp. In most instances in

this pilot work, reasonable waveforms were obtained using the method described above.

A3.3.8. In order to further reduce impedance, electrodes had to be filled with an electrolyte gel. This was normally kept under refrigeration. Prior to testing, it was allowed to reach room temperature. The usual technique employed with adult subjects of gel insertion via a syringe and hollow blunted needle was modified for use with infants. The prime concern in developing an infant electrode filling technique was to avoid any remote possibility of injury to the subjects. For this reason, the needle was made safer still by fitting it with a disposable plastic guard fitted to the end of the needle. Because of the need for ultra caution, a fresh, sterile needle was used for each subject. This was to preclude any slight risk of cross-infection. During the briefing with the mother before testing, the safety of this arrangement was emphasised. Parents were always allowed to handle the needle and syringe. In this way, they could ensure its safety for themselves. Furthermore, the experimenter would attempt to scratch himself with a demonstration needle and syringe, as a graphic demonstration of its safety.

A3.3.9. During subsequent experimental work, no attempt was made to check skin impedances during headcap fitting. Early experience had rapidly shown that, even if they were found to be high, there was no safe remedy for reducing them. With adult subjects, abrading of the skin with the filling needle tip would be used to help reduce impedances. This would clearly not be permissible with infants in an experimental setting. Hence any impedance reduction technique was at best a compromise between satisfactory impedances and loss of subject compliance.

A3.3.10. However, the problem of relatively high skin impedances with infants is not confined just to the BEAM technique. Indeed, it is a problem with more traditional forms of infant EEG measurement, as stated by Kagawa (1962):

> " ... *it is often difficult to obtain a low DC resistance even with the most carefully applied electrodes*".

Hence the method used was a compromise between obtaining reasonably low skin impedances and the avoidance of any skin damage.

Section 3: Stimuli

A3.4.1. Baby food odour stimuli were chosen for the following reasons. Firstly, such odours would be perceived as 'natural' by parents and hence harmless to their infants. Odour stimuli for the study were therefore obtained following approaches to all the major European baby food manufacturers. Of these, Cow & Gate Limited agreed to donate samples for experimental use. This company has been a household name in the UK for many years, with a reputation for high quality. Because of this, the stimuli were likely to be acceptable to parents in terms of safety of exposure to their child. Commercially available baby foods from the same manufacturing batch are very similar in composition, with comparable odours.

A3.4.2. Secondly, food odours are an everyday experience in homes and hence have a high degree of 'ecological validity' to babies. In other words, they might represent 'biologically significant' odours, as argued in Chapter 3. Thirdly, many of the babies would have begun weaning and had some experience of food odour at close range. This slight familiarity might reduce the possibility of 'startle' response due to very novel odours.

A3.4.3. The technique for preparing the odours, with regard to 'head space', is described in the text of Chapter 4. No technical problems were encountered with using this method of odour preparation and presentation. However, facilities for measuring the chemical profile of the 'head space' were not available in the laboratory. Hence the concentration of odours in the 'head space' has to be inferred rather than given an exact quantity.

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